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Short Communication

Analysis of NTRK expression in gastric and esophageal adenocarcinoma (AGE) with pan-TRK immunohistochemistry

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ABSTRACT

Small molecule inhibitors such as Larotrectinib have been recently approved in the treatment of patients with fusions of the neurotrophic-tropomyosin receptor tyrosine kinase (NTRK) genes 1-3. These genomic rearrangements have been reported across different tumor subtypes with a high prevalence in rare tumors. However, in gastric and esophageal adenocarcinoma (AGE) NTRK fusions have also been described in a subset of Asian patients. In order to study the prevalence of this alteration in Caucasian patients with AGE we performed immunohistochemistry for pan-NTRK in 438 formalin-fixed paraffin embedded (FFPE) tumor samples. While we found NTRK expression in gastric glands and tumor adjacent nerve tissue, we did not detect this marker in the tumor compartment. Based on our findings NTRK fusions do not seem to play a role in the molecularpathology of Caucasian AEG patients, so that other treatment options are required.

1. Introduction

Under physiologic conditions the neurotrophic-tropomyosin receptor tyrosine kinases (NTRKs 1–3) play a pivotal role in the embryologic development process of the central nervous system [7]. However, NTRK gene fusions lead to oncogenic transformation by activating downstream cell growth and proliferative pathways. These genomic rearrangements show a high prevalence in rare tumors (e.g. mammary-analog secretory carcinoma of the salivary gland, secretory breast carcinoma, and infantile congenital fibrosarcoma), while they are rarely found (1% or less) in common cancer types [15]. Nevertheless, recent trials, which demonstrated the efficacy of Larotrectinib in *NTRK* fusion-positive cancer, underline the importance of this pathway [3]. Moreover, in November 2018 the Food and Drug Administration approved Larotrectinib for the treatment of patients with advanced solid tumors harboring an *NTRK* gene fusion, irrespective of the tumor tissue origin [17]. As an FDA-approved test for the detection of *NTRK* gene fusion is not available so far, different techniques, including next-generation sequencing (NGS), fluorescence-in-situ-hybridization (FISH) and immunohistochemistry (IHC), are applied to determine direct or indirect alterations of the *NTRK* genes [9]. However, the current ESMO recommendations include pan-TRK immunohistochemistry as a possible method to screen for *NTRK* gene fusions [8].

In order to determine the prevalence of *NTRK* gene fusions in Caucasian patients with gastric and esophageal adenocarcinoma (AGE), which remains a disease with dismal prognosis and is potentially targetable with Larotrectinib, we tested a cohort of 438 patients with pan-TRK immunohistochemistry.

2. Materials and methods

Clinical data from 438 patients with AGE of all tumor stages, primarily treated by surgery between 1992 and 2004 at the Charité – Universitätsmedizin Berlin, were collected retrospectively. The mean follow-up was 121.7 months (95% CI: 113.9–129.5). Overall survival, used as a measure of prognosis, was defined as time from diagnosis to

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death or last follow-up. The data including patient characteristics and follow-up information were retrieved from the patient management software (SAP) and the regional population-based cancer registry ("Gemeinsames Krebsregister"). This study was approved by the Institutional Review Board of the Charité (EA4/115/10).

Tissue samples were collected from the archive of the department of pathology, Charité- University Medicine Berlin. Formalin-fixed paraffin embedded (FFPE) tumor samples were available from surgically treated chemotherapy-naive patients. All samples were reevaluated according to histological diagnosis, tumor stage and grade, and classified by the histological architecture of AGE/S carcinoma using Lauren's and Ming classification by a specialist for gastrointestinal pathology (M.W.). Data concerning tumor size, depth of invasion, and tumor invasion of veins or lymphatic vessels were retrieved from the Charité - University Medicine patient management software. Tissue samples were screened in a HE-stained section for representative areas of solid tumors. Two 1 mm-diameter tissue cores were punched out from each of the 438 available cases and were transferred to a recipient paraffin block. Afterwards, 4 µm sections were consecutively cut from each tissue microarray block. HE staining was performed on tissue micro array (TMA) sections for reconfirmation of content of tumor and non-tumor tissue in each core.

Pan-TRK (TRK A, B and C) expression was detected using a monoclonal anti panTRK antibody (clone: EPR17341, Abcam, Cambridge, UK) at 1:50 dilution after heat-mediated antigen retrieval using epitope retrieval solution pH 8.0 (Novocastra laboratories, Newcastle upon Tyne, UK). Primary antibody detection and visualization were done using ImmPress anti rabbit IgG reagent (Vector) and diaminobenzidine (DAB+, Dako, Glostrup, Denmark). Nuclei were visualized using Gill's formula hematoxyline counterstaining (Vector laboratories, Burlingame, CA, USA). Appropriate positive controls (appendix for panTRK) were included in the staining runs.

3. Results

Clinical characteristics of the analyzed cohort are depicted in Table 1. In the analyzed cohort (438 patients) we were not able to detect any expression of NTRK in the tumor compartment irrespective of the clinical situation, type of sample or the tumor localization. Adjacent nerve tissue stained strongly for NTRK serving as internal positive control (Fig. 1). Interestingly, we found cytoplasmatic expression of NTRK within gastric glands, in line with the data available in the Human Protein Atlas [14].

Table 1
Clinical characteristics.

Characteristic	No. of patients (n = 438)	%
Gender		
Female	178	40.6
Male	260	59.4
Age		
< 65 years	244	55.7
> 65 years	194	44.3
Localization		
esophageal	66	15.1
gastric	372	84.9
UICC		
I	162	37.0
II	111	25.3
III	50	11.4
IV	115	26.3
Lymphatic Infiltration		
L0	150	34.2
L1	213	48.6
Unspecified	75	17.1
Venous Infiltration		
V0	233	53.2
V1	120	27.4
Unspecified	85	19.4
Lauren Classification		
Intestinal	176	40.2
Diffuse	201	45.9
Mixed	58	13.2
Unspecified	3	0.7
Ming Classification		
Expansive	172	39.3
Infiltrative	259	59.1
Unspecified	7	1.6
Grading		
G1	8	1.8
G2	116	26.5
G3	311	71.0
Unspecified	3	0.7

4. Discussion

Based on the findings of recent clinical trails the FDA approved Larotrectinib for the treatment of patients with advanced solid tumors harboring an *NTRK* gene fusion [3]. Given the dismal prognosis of patients with gastric and esophageal adenocarcinoma (AGE) Larotrectinib might be a potential treatment option.

In the given study we used pan-TRK antibodies to screen for NTRK fusions as immunhistochemical survey for NTRK protein expression was shown as a sensitive method, which is inexpensive, available in most laboratories and requires a short turnaround time [2]. Due to the following points this method comprises caveats, which represent a limitation of our study. The disadavantages in general include the lack of a standardized scoring system and additional testing in a positive case [5]. Moreover, new investigations have demonstrated varying expression performances for different pan-TRK antibody clones [1]. Especially 2 recent large scale studies, which characterized NTRK fusions, indicate that pan-TRK antibody shows lower sensitivity for NTRK3 fusions [4,12]. However, Solomon et al. also demonstrated that sensitivity for NTRK detection depended on tumor type. Interestingly, sensitivity of 80% was shown for sarcomas while it reached 100% in inflammatory myofibroblastic tumor, appendiceal adenocarcinoma, cholangiocarcinoma, glioma, and melanoma [12]. Furthermore, other detections methods of *NTRK* fusion genes show limitations as well. FISH testing may report false negative results if the breakpoints involve noncanonical sites [10]. While NGS is able to detect novel fusion partners and fusion genes expressed at RNA level, it requires adequate tumor purity for DNA-NGS and high RNA quality for RNA-NGS [11]. These facts lead to a widely accepted two-step diagnostic algorithm, in which an initial immunohistochemical survey is followed by an independent verification (NGS or FISH) method in positive tested cases [8]. However, cases without NTRK expression are regarded as negative and do not require further molecular validation.

According to the ESMO recommendations we applied pan-TRK antibodies to screen for *NTRK* gene fusions in the given AGE cohort. However, we could not detect any NTRK expression in the carcinoma compartment, while tumor adjacent nerve tissue served as positive controls. In contrast, previous investigations, which were all conducted in Asia, showed an expression of NTRK ranging from 20 to 50% of the studied cohorts [6,13]. Moreover, Kamiya et al. demonstrated that NTRK was associated with tumor progression and poor survival in gastric cancer patients

Similar to the study by Westphalen et al., who analyzed the expression of NTRK in biliary tract cancer, we could not detect *NTRK* gene fusions in our large cohort of AGE [16]. Comparable to this study our cohort included only Caucasian patients, whose clinical outcomes and tumor biology differs from Asian patients especially for gastrointestinal cancer

In conclusion, our and other data underline the molecular differences in gastrointestinal cancer between Asian and Caucasian patients. Moreover, we do not find *NTRK* gene fusions in Caucasian AEG patients, so that other therapeutic strategies are necessary.

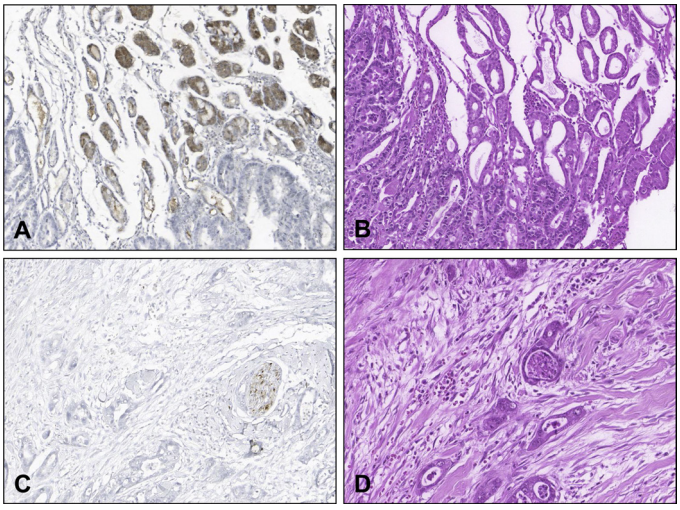

Fig. 1. A: NTRK Expression in gastric glands, adjacent adenocarcinoma component without NTRK expression. B: Corresponding HE staining highlightning the adenocarcinoma invasion front. C: NTRK Expression in nerve tissue. D: Corresponding HE staining showing perineural invasion.

Notes

Compliance with ethical standards

Research involving human participants

Informed consent was obtained from all individual participants included in the study.

All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions.

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Authors' contribution

AA: Design of the study, data analysis, writing of the manuscript; CD: Data collection, intellectual input; MvW: data collection, critical discussion of the manuscript; BR: provided patient materials (TMA) and patient data; US: study design, data collection, data analysis; DH: intellectual input, critical discussion of the manuscript, EB: construction of TMA; TMA staining, MH: intellectual input, critical discussion of the manuscript, CT: study design, project management, writing and discussion of the manuscript.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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