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ERBB2 mutation: A promising target in non-squamous cervical cancer

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HIGHLIGHTS

- Somatic ERBB2 mutations were detected in 3.15% (32 of 1015) patients.
- The ERBB2 mutation was significantly increased in non-squamous carcinoma.
- 18.75% of the ERBB2-mutant patients harbored concurrent PIK3CA or KRAS mutations.
- The ERBB2 overexpression/amplification rate was 3.82% in cervical cancer.

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ABSTRACT

Objective. ERBB2 mutations have been found in a subset of invasive cervical cancer (ICC). Nevertheless, the prevalence, mutation spectrum, clinicopathological relevance, human papillomavirus (HPV)-genotype association and prognostic significance of ERBB2-mutated ICCs have not been well established.

Methods. In this study, ICC samples ($N = 1015$) were assessed for mutations in ERBB2, KRAS, and PIK3CA by cDNA-based Sanger sequencing.

Results. Somatic ERBB2 mutations were detected in 3.15% patients. The ERBB2 mutation rate was significantly higher in adenocarcinoma (4.52%, 7/155), adenosquamous carcinoma (7.59%, 6/79) and neuroendocrine carcinoma (10.34%, 3/29) than that in squamous carcinoma (2.14%, 16/749) ($P = 0.004$, Fisher exact test). In addition, 18.75% of the patients carrying ERBB2 mutations concomitantly harbored PIK3CA or KRAS mutations. Patients with ERBB2-mutated ICCs tended to have a worse prognosis than those with wild-type or PIK3CA-mutated ICCs but a better prognosis than those with KRAS-mutated ICCs.

Conclusions. This study provided a promising rationale for the clinical investigation of tyrosine kinase inhibitors for the treatment of cervical cancer with ERBB2 mutations. Patients with non-squamous cell carcinomas have priority as candidates for ERBB2-targeted therapy. Concurrent PIK3CA/RAS mutations should be considered in the design of clinical trials.

Abbreviations: ICC, invasive cervical cancer; HPV, human papillomavirus; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; TKIs, tyrosine kinase inhibitors.

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1. Introduction

Although the incidence rates of cervical cancer have decreased dramatically in developed areas as a result of effective screening, cervical cancer remains a serious issue for women worldwide, particularly in developing countries [1]. There are limited efficient therapeutic options for metastatic or recurrent carcinoma of the uterine cervix. The overall response rate of platinum-based chemotherapy in these patients is 20 to 52% [2,3]. Incorporating bevacizumab into the chemotherapy regime improved the median overall survival and progression-free survival by approximately four and two months, respectively [4]. Therefore, a new treatment strategy is urgently required.

We examined mutations in 16 targetable oncogenic genes using reverse transcription polymerase chain reaction and direct sequencing in 285 cervical cancer patients [5]. Ninety-two nonsynonymous mutations were identified in 29.8% of the patients and the top three gene mutations were listed as follows: PIK3CA (12.3%), KRAS (5.3%) and ERBB2 (4.2%). By expanding sample size, we further investigated the mutation rate and clinical implication of PIK3CA [6] and KRAS [7]. The current study aimed to explore the ERBB2 mutation profile and clinical correlation in a larger population.

As a member of the human epidermal growth factor receptor family, ERBB2 (HER2) is a trans-membrane protein composed of an extracellular domain (ECD), a trans-membrane domain (TM), and an intracellular tyrosine kinase domain (KD). Somatic mutations of ERBB2 have been identified in a wide range of solid tumors, including breast, lung, colorectal, bladder, and gastric cancers [8–12]. Specific ERBB2 mutations are clustered in exon 8 of the kinase domain or exon 19–21 of the extracellular domain, and the majority of ERBB2 somatic mutations are activating mutations that likely drive tumorigenesis [11–13]. Preclinical data have demonstrated that tyrosine kinase inhibitors (TKIs) of ERBB2 are effective in breast, lung, and colorectal cancers harboring activating mutations of ERBB2 [11–13]. Several inhibitors have been developed to treat HER2-driven cancers, including afatinib, neratinib and lapatinib. More than 100 clinical trials of ERBB2-targeted TKIs have been registered at https://clinical trials.gov [14]. Increasing clinical data - indicate that ERBB2-targeted TKIs improve the outcome of cancers bearing ERBB2 mutations when administered as monotherapy or combined with chemotherapy [15–17]. In brief, ERBB2 mutations have emerged as promising therapeutic targets in a variety of human cancers.

Oncogenic mutations of ERBB2 have been found in a subset of invasive cervical cancer (ICCs) since 2014 [5,18,19]. However, according to database of Catalogue of Somatic Mutations in Cancer (COSMIC) [20] and The Cancer Genome Atlas (TCGA) [21], less than four hundred cervical samples have been tested for ERBB2 mutations so far. In addition, specific characteristics of ERBB2 mutations in cervical cancer remain to be established, including the prevalence, mutation spectrum, clinicopathological features, HPV-genotype correlation, prognostic implication, and related genetic background information of ERBB2-mutated ICCs, which are of paramount importance for designing clinical trials of ERBB2-targeted TKIs for cervical cancer patients.

2. Materials and methods

2.1. Patients and specimens

Ethical approval was granted by the Ethics Committee of Fudan University Shanghai Cancer Center (NO.050432-4-1212B). Informed consent was obtained from each participant. The inclusion criteria of the patients, preparation of samples and clinical data retrieval were performed as described in our previous publications [5,6]. Patients included fulfilled the following criteria: pathology confirmed disease, FIGO stage IB1-IIA2 and no preoperative treatment. The tumor specimens were obtained during surgery. A cohort of 1015 cervical cancer patients was subjected to ERBB2 mutational analysis in this study. Amongst these patients, 157 cases were also tested for HER2 overexpression/amplification. The tumor samples were collected from January 2010 to December 2014.

2.2. HPV genotyping

HPV 16- and 18-specific LCR/E6/E7-based real-time PCR was used for the detection of the two most common high-risk HPV types. The reactions, primers, and probes are presented in Supplementary Table S1. Samples with threshold cycle (Ct values) <35 were considered HPV positive.

2.3. Mutation analysis

ERBB2, PIK3CA and KRAS mutation analyses were performed using cDNA-based Sanger sequencing as previously described [5]. The primers are presented in Supplementary Table S2.

2.4. Immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) analysis

HER2 overexpression was detected by IHC staining on microarray slides using anti-HER-2/NEU (4B5) antibody (Ventana Medical Systems, Inc. Tucson, Arizona). The slides were independently assessed by two pathologists according to the scoring system of breast cancer [22]. HER2 amplification was tested by FISH using thePathVysion®HER2 DNA Probe kit. The FISH slides were scored by two pathologists without prior knowledge of the IHC scores. Cases with IHC (+++) and IHC (++)/FISH (+) were classified as HER2 positive.

2.5. Statistical analysis

The comparison between groups was performed by the Student's *t*-test, Pearson's Chi-Square test, or Fisher's exact test. Survival curves were assessed using the Kaplan-Meier method and the log-rank test. The Statistical Package for Social Science (SPSS) (Version 19.0, SPSS, Inc., Chicago, IL, USA) was used for the analyses.

3. Results

3.1. ERBB2 mutations detected in ICC

For the entire cohort, HPV 16 and 18 positivity accounted for 55.27% (561/1015) and 18.72% (190/1015), respectively. Amongst the 1015 ICCs examined, 33 non-synonymous somatic mutations of ERBB2 were detected in 32 patients (3.15%). The mutations included 30 missenses and 3 in-frame deletions. In-frame insertions of exon 20, which are enriched in lung adenocarcinomas, were not found in ICCs in this series [23]. Nineteen ERBB2 mutations were located within the ECD, four in the TM, and 10 in the KD. The most prevalent mutation spot was S310F (6 cases), followed by A270S (5 cases). The mutation spectrum of ERBB2 in ICC is presented in Fig. 1.Details of the 32 ERBB2 mutant ICCs are presented in Table 1. Interestingly, one patient concomitantly harbored two mutations: A270S and N764 K.

Non-synonymous somatic mutations of PIK3CA and KRAS were detected in 146 (14.38%) and 33 (3.25%) patients, respectively. Four of the 32 patients (12.5%) carrying ERBB2 mutations concomitantly harbored KRAS mutations, and two of these 32 patients (6.25%) harbored PIK3CA mutations.

3.2. Clinicopathological characteristics of the patients with ERBB2 mutations

The clinicopathological characteristics of the patients with ERBB2 mutations are presented in Table 2. The prevalence of ERBB2 mutations was significantly higher in adenocarcinoma (4.52%, 7/155), adenosquamous carcinoma (7.59%, 6/79) and neuroendocrine

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