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Original Article

Mutational analysis of *KRAS* and its clinical implications in cervical cancer patients

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ABSTRACT

Objective: The predictive and prognostic role of *KRAS* mutations in cervical cancer remains inconclusive. The aim of this study was to explore the clinicopathological and prognostic relevance of *KRAS* mutations in invasive cervical cancers (ICC).
Methods: Reverse transcription polymerase chain reaction (PCR) and Sanger sequencing were employed to detect *KRAS* mutations in 876 ICC patients. Quantitative real-time PCR was used to detect human papillomavirus (HPV) 16 and HPV 18.
Results: Non-synonymous mutations of *KRAS* were identified in 30 (3.4%) patients. These mutations were more common in non-squamous cell carcinoma than in squamous cell carcinoma (SCC) (8.2% vs. 2.2%, respectively, p<0.001) and were associated with HPV 18 infection (p=0.003). The prevalence of mutations was highest (18.2%) in the uncommon histological subtypes followed by adenocarcinoma (AC, 7.3%) and adenosquamous carcinoma (ASC, 5.8%). During the median follow-up of 55 months, compared to patients with wild-type *KRAS*, a greater percentage of patients with mutant *KRAS* relapsed (20.0% vs. 42.9%, respectively, p=0.007). The 3-year relapse-free survival was poorer in patients with mutant *KRAS* than in patients without *KRAS* mutations (57.1% vs. 81.9%, respectively, p=0.001). Furthermore, the multivariate analysis showed that the presence of a *KRAS* mutation was an independent predictor for disease recurrence (hazard ratio [HR]=2.064; 95% confidence interval [CI]=1.125–3.787; p=0.019).
Conclusion: *KRAS* mutations were predominant in non-SCCs of the cervix and were associated with HPV 18 infection. A combination of *KRAS* mutation detection and HPV genotyping would be useful in identifying patient with poor prognosis for further interventions.

Keywords: *KRAS*; Uterine Cervical Neoplasms; Papillomaviridae; Prognosis

INTRODUCTION

In China, cervical cancer is the eighth leading cause of cancer-related death among women and is responsible for more than 20,000 deaths annually [1]. Despite improvements in cervical cancer screenings and treatments over the past 50 years, the incidence and mortality rates of cervical cancer in China have increased annually since 2000 [2]. Treatment of advanced or

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Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Author Contributions

Conceptualization: J.W., X.L., W.X., Y.H.; Data curation: J.W., X.L., P.X., H.T., S.X., Y.H.; Formal analysis: J.W., X.L., Y.H.; Funding acquisition: Y.H., X.L.; Investigation: J.W., Y.H.; Methodology: J.W., X.L., S.X., H.T., P.X.; Project administration: Y.H., X.L.; Resources: W.X., Y.H.; Software: J.W., P.X.; Supervision: Y.H., W.X.; Validation: W.X., Y.H.; Visualization: J.W., X.L., H.T., S.X.; Writing - original draft: J.W.; Writing - review & editing: Y.H., X.L.

recurrent cervical cancer is still limited, resulting in poor survival [3]. Targeted therapy based on oncogenic mutations might be a potential approach to improve treatment outcomes.

The *KRAS* protein functions as a GTPase and plays a vital role in regulating cell differentiation, proliferation, and survival [4,5]. Somatic *KRAS* mutations can be detected in approximately 30% of all human cancers [6]. The three most common residues for *KRAS* mutations—G12, G13, and Q61—are responsible for intrinsic and GAP-induced GTP hydrolysis; point mutations at these residues can lead to the accumulation of cellular GTP-bound *RAS*, which activates downstream signaling pathways [6]. *KRAS* mutations have been confirmed as a promising prognostic marker in non-small cell lung cancer (NSCLC) and colorectal cancer (CRC) [7-9]. In a large cohort of patients with CRC, the multicenter Refractory Angina Spinal Cord stimulation and usuAL care (RASCAL) study demonstrated that the *KRAS* G12V mutation exerted more aggressive properties than other *KRAS* mutations regarding disease recurrence and death [10,11]. In *BRAF* wild-type CRC, Imamura reported that a mutation at *KRAS* codon 12 but not at *KRAS* codon 13 was associated with reduced survival [12]. Thus, different *KRAS* mutations may have distinct clinical responses.

To date, *RAS* proteins have not yielded any successful targeted therapies and have been viewed as "undruggable" for many years. Some drugs have been designed to block pathways downstream of *RAS*, such as RAF, MAPK-MEK, and ERK; however, their efficacy has been generally disappointing [13-15]. Nevertheless, the patient's *KRAS* mutation status has been confirmed to be a criterion for implementing treatment with anti-epidermal growth factor receptor (EGFR) antibodies [16], as this treatment modality is more successful in patients with *RAS* wild-type metastatic CRC than in patients with mutant *RAS*. The combination of a MEK inhibitor and a fibroblast growth factor receptor 1 (FGFR1) inhibitor leads to tumor cell death in *KRAS*-mutant lung cancer cells but not in corresponding *KRAS* wild-type cells [17]. Thus, detecting *KRAS* mutations has been shown to be useful in selecting patients who could benefit from some of the available targeted treatments.

KRAS mutation has been identified as the second common oncogenic mutation following *PIK3CA* in our previous comprehensive analysis of 16 targetable oncogenic mutations in 285 cervical cancers [18]. In this study, a larger cohort of patients with cervical cancer (876 patients) were enrolled to explore its association with clinicopathological characteristics and prognosis over a longer follow-up period as well as to determine the relevance of human papillomavirus (HPV) infection in the incidence of *KRAS* mutations.

MATERIALS AND METHODS

1. Patient data

This study was approved by the Ethics Committee at the Fudan University Shanghai Cancer Center (FUSCC 050432-4-1212B) and was conducted in accordance with the approved guidelines. Patients with cervical cancer were enrolled between January 1, 2010 and December 30, 2012 if they satisfied the following conditions: pathologically determined primary cervical carcinomas, stages IB1–IIA2 disease according to the 2009 International Federation of Gynecology and Obstetrics (FIGO) staging system, and no prior neoadjuvant chemotherapy or radiation. Cervical tumor specimens were collected during either radical hysterectomy or trachelectomy procedures and stored at −80°C in RNAlater solution (Ambion; Thermo Fisher Scientific, Waltham, MA, USA). After the specimens were assessed

by 2 independent pathologists (Xuxia Shen and Wentao Yang), those with either insufficient tumor material for a comprehensive mutational analysis or fewer than 50% malignant cells within the entire tissue sample were excluded. In total, 876 patients were eligible for this study. Among these, 553 patients received adjuvant therapy after surgery according to the guidelines, including 64 patients who received pelvic radiotherapy alone, 68 patients who received chemotherapy alone and 421 patients who received concurrent chemoradiation with or without subsequent systemic chemotherapy. The specific clinicopathological characteristics, including age, menopausal status, histological type, tumor size, depth of myometrial invasion, lymphovascular space involvement (LVSI), regional lymph node metastasis, parametrial involvement, and distant metastasis, were recorded. The patients were followed up for disease recurrence and survival duration either in the clinic or by telephone. All patients provided written informed consent for the analysis of their tumor specimens and the collection of clinical information.

2. Detection of *KRAS* mutations

Genomic DNA and total RNA were extracted from the tumor tissues using a DNA/RNA isolation kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. cDNA was obtained by reverse transcribing 2 μg of total RNA using an M-MLV Reverse Transcriptase kit (Invitrogen, Waltham, MA, USA) and was used for mutational analysis and HPV detection. Mutational analyses were conducted according to our previous protocol [18]. *KRAS* (exons 1–4) was amplified using KOD-Plus-Neo DNA polymerase (Toyobo, Tokyo, Japan) with the following primers: *KRAS*-F:CCATTTCGGACTGGGAGCGA, and *KRAS*-R: GGCATCATCAACACCCA GAT. The polymerase chain reaction (PCR) products were directly sequenced using the Sanger sequencing technique, and all mutations were confirmed by an additional independent PCR experiment.

3. Quantitative real-time PCR assay for the detection of HPV

A TaqMan quantitative real-time PCR assay was used to detect HPV 16 and HPV 18 [19] using the following primers: HPV 16 (F: 5'GAACCGAAACCGGTTAGTATAA 3', R: 5'ATGTATAGTTGTTTGCAGCTCTGT3') and HPV 18 (F: 5'GGACCGAAAACGGTGTATATAA 3', R: 5'CAGTGAAGTGTTCAGTTCGGT 3'). The probes for HPV 16 and HPV 18 were CATTTTATGCACCAAAAGAGAACTGCAATGTTTC and ATGTGAGAAACACACCACAATACTATGGCGCG, respectively. The PCR reaction (10 μL) comprised 5 μL of Premix Ex Taq™; 1 μL of Primer Mix (10 μM); 1 μL of Probe Mix (40 nM for HPV 16, 200 nM for HPV 18); 1 μL of sample cDNA and 2 μL of dH2O. The PCR was performed on an ABI 7500 instrument (Applied Biosystems, Foster City, CA, USA) as follows: denaturation step at 95°C for 30 seconds and 40 cycles of 5 seconds at 95°C, 10 seconds at 55°C, and 20 seconds at 72°C.

4. Statistical analysis

All statistical analyses were performed using IBM SPSS statistics software, version19 (IBM Corporation, New York, NY, USA). Either the χ^2 test or Fisher's exact test was used to analyze the association between *KRAS* mutations and the patients' clinicopathological characteristics. Relapse-free survival (RFS) was defined as the period from the completion of surgery to the date of documented evidence of disease recurrence. The end of the observation period was March 31, 2016, and patients without disease recurrence were censored at their last follow-up visit. Survival curves were calculated using the Kaplan-Meier method, and differences between the groups were tested using the log-rank test. The Cox proportional hazards model was used for multivariate survival analysis. Statistical significance was set at $p<0.050$.

RESULTS

1. Characterization of *KRAS* mutation in cervical cancers

Among 876 patients, 30 non-synonymous mutations of *KRAS* were identified (3.4%), the majority (86.7%, 26/30) of which were found on exon 2. Ten percent (3/30) of the mutations were located on exon 3, and only one (3.3%) mutation existed on exon 4. The detailed mutation and clinicopathological information were provided in Supplementary **Table 2**. **Fig. 1** demonstrated the distribution of the mutation sites in *KRAS*-mutant carcinomas. The G12 residue on *KRAS* was the most frequently mutated (17/30, 56.7%) followed by G13 (4/30, 13.3%). *KRAS* G12 mutations are predominant in non-squamous cell carcinomas (SCCs) (73.3%), and the rates of *KRAS* G12 & G13 mutations in SCC were 40% and 20%, respectively. A mutation of residue Q61 was only found in one patient in this cohort despite its status as a common mutation of *KRAS* in other human cancers [6]. In addition, 3 novel *KRAS* mutations (G15C, S39Y, and F156Y), which have not been previously described in cervical cancer according to the Catalogue of Somatic Mutations in Cancer (COSMIC) database (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic; Jun 8, 2017), were identified in our patient cohort.

2. Clinicopathological association of *KRAS* mutations

Table 1 summarizes the association between *KRAS* mutations and the patients' clinicopathological characteristics. *KRAS* mutations were more common in non-SCC than in SCC (8.2% vs. 2.2%, p<0.001). The highest prevalence of mutations (18.2%) occurred in uncommon histological subtypes (neuroendocrine carcinoma, clear cell carcinoma, carcinosarcoma, and poorly differentiated carcinoma) followed by adenocarcinoma (AC; 7.3%), adenosquamous carcinoma (ASC; 5.8%), and SCC (2.2%) (p<0.001, **Table 1**).

Either HPV 16 or HPV 18 was detected in 631 patients (71.9%), with 487 (55.6%) patients positive for HPV 16, 136 (15.5%) patients positive for HPV 18, and 8 (0.9%) patients positive for both. HPV 18 positive patients were more likely to harbor a *KRAS* mutation than either HPV 16 positive or negative patients (8.1% vs. 2.1% vs. 3.7%, respectively, p=0.003, **Table 1**). *KRAS* mutations were not found to correlate with other clinicopathological characteristics such as lymph node metastasis, larger tumor size, deep myometrial invasion and the presence of LVSI.

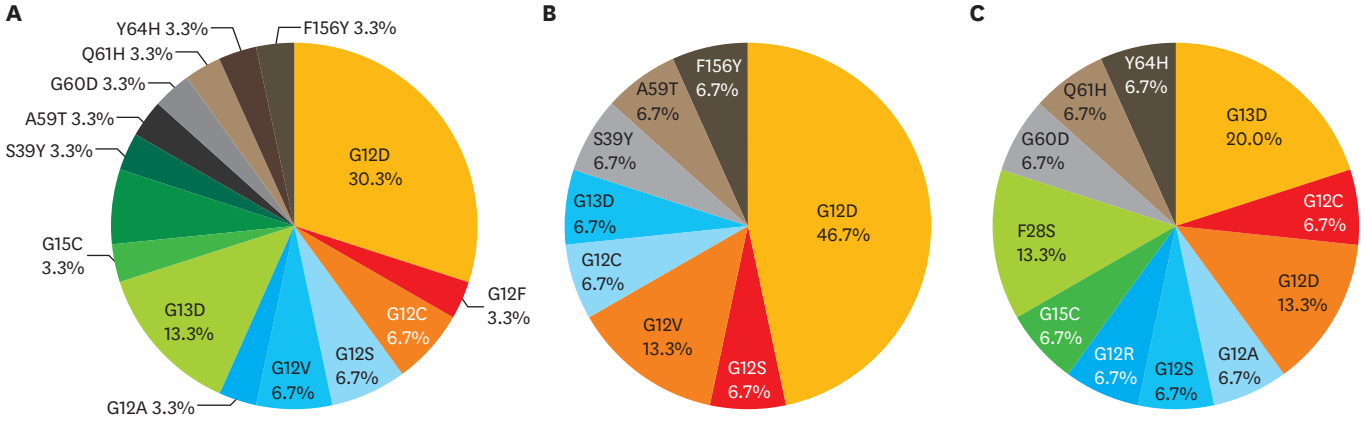

Fig. 1. Identification of *KRAS* mutation hotspots in cervical cancers. (A) *KRAS* mutations identified in 30 cervical cancers. (B) *KRAS* mutations identified in 15 non-SCCs. (C) *KRAS* mutations identified in 15 SCCs.
SCC, squamous cell carcinoma.

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Table 1. Association between *KRAS* mutations and clinicopathological parameters

Variables	Cases	*KRAS* mutation status		p-value (χ^2 test)
		Wild-type (n=846)	Mutant (n=30)	
Age (yr)				0.664
<47	404	389	15	
≥47	472	457	15	
Menopausal status				0.688
Premenopausal	556	538	18	
Postmenopausal	320	308	12	
HPV infectious*				0.003
HPV 16	487	477	10	
HPV 18	136	125	11	
HPV 16 & 18	8	8	0	
Negative	245	236	9	
Histological subtypes				<0.001
SCC	693	678	15	
AC	109	101	8	
ASC	52	49	3	
Others†	22	18	4	
FIGO stage				0.673
IB	434	418	16	
IIA	442	428	14	
Node status				0.525
Negative	629	609	20	
Positive	247	237	10	
Tumor sizes (cm)				0.698
>4	264	254	10	
≤4	612	592	20	
Depth of myometrial invasion				0.745
Whole thickness	279	268	11	
>1/2	349	339	10	
≤1/2	248	239	9	
LVSI				0.097
Yes	331	324	7	
No	545	522	23	
Parametrial involvement				0.081‡
Yes	49	45	4	
No	827	801	26	

AC, adenocarcinoma; ASC, adenosquamous carcinoma; FIGO, International Federation of Gynecology and Obstetrics; HPV, human papillomavirus; LVSI, lymphovascular space involvement; SCC, squamous cell carcinoma.
*Eight Cases positive for both HPV 16 & 18 were excluded in χ^2 test. All the 8 cases were negative for mutations. †Others include neuroendocrine carcinoma (16), clear cell carcinoma (2), carcinosarcoma (2), and poorly differentiated carcinoma. ‡Fisher's exact test was used.

3. The association between *KRAS* mutation and treatment outcome

A total of 767 (87.6%) patients were included in the survival analysis with a median follow-up duration of 55 months (range: 1–75 months). Disease recurrence was documented in 160 patients (20.9%) during the follow-up intervals, with 12 (42.9%) of 28 patients with mutant *KRAS* experiencing recurrence during follow-up; this rate was significantly higher than rate of patients with wild-type *KRAS* (20.0%, 148/739, p=0.007; Fisher's exact test). Detailed recurrence information was available for 759 patients. Distant metastasis outside of the pelvis was documented in 113 (14.9%) patients, and pelvic recurrence was documented in 39 patients (5.1%). In patients with a *KRAS* mutation, distant metastasis and pelvic recurrence within the surgical or radiation area were documented in 29.6% and 11.1% of the patients, respectively; these rates were significantly higher than those in patients with wild-type *KRAS* (14.3% and 4.9%, respectively, p=0.023). Furthermore, a significant relation was found between *KRAS* mutation and distant metastasis (p=0.016), but not for local recurrence (p=0.101) (**Supplementary Table 1**).

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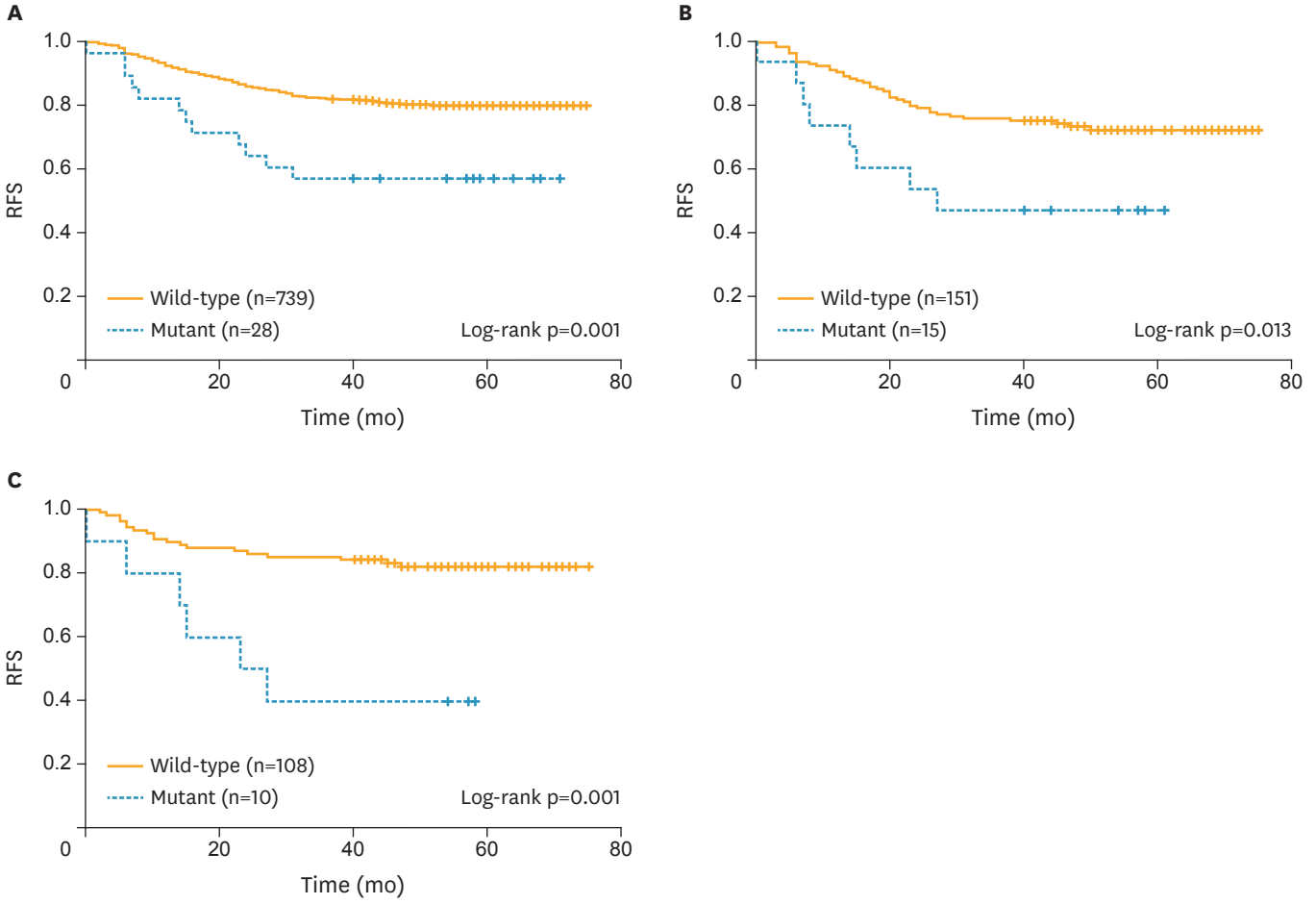

Fig. 2. Kaplan-Meier curve of RFS. (A) Kaplan-Meier curve of RFS of the 767 cervical cancer patients. (B) Kaplan-Meier curve of RFS of the 166 patients with non-SCCs. (C) Kaplan-Meier curve of RFS of the 118 patients with HPV 18 infections.
HPV, human papillomavirus; RFS, relapse-free survival; SCC, squamous cell carcinoma.

KRAS mutations were confirmed to be associated with patient survival in both univariate and multivariate analyses. The 3-year RFS of patients with mutant *KRAS* was significantly poorer than that of patients with wild-type *KRAS* (57.1% vs. 81.9%, respectively, p=0.001) (**Fig. 2A**). The multivariate analyses revealed that *KRAS* mutations were an independent predictor for worse RFS (hazard ratio [HR]=2.064; 95% confidence interval [CI]=1.125–3.787; p=0.019) (**Table 2**).

Furthermore, survival analysis was performed in patients with SCC or non-SCC. Among the 166 patients with non-SCC, the 3-year RFS was significantly poorer in patients with a *KRAS* mutation than in those with wild-type *KRAS* (46.7% vs. 75.5%, p=0.013) (**Fig. 2B**); however, this finding was not replicated in patients with SCC (**Supplementary Fig. 1**).

Among patients positive for HPV 18, those with a *KRAS* mutation had a shorter survival than patients with wild-type *KRAS* (3-year RFS: 40.0% vs. 85.2%, respectively, p=0.001). However, an association between *KRAS* mutations and RFS was not observed in patients positive for HPV 16 (p=0.478) (**Fig. 2C**).

In addition, the 3-year RFS was compared among 4 patients with *KRAS* G13 mutations and 17 patients with *KRAS* G12 mutations. A worse survival trend was revealed in patients harboring

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Table 2. Identification of predictors for disease-relapse survival by univariate and multivariate analysis in 767 patients with cervical cancer

Variables	Univariate analyses			Multivariate analyses		
	HR	95% CI	p-value	HR	95% CI	p-value
Age (≥47 yr vs. <47 yr)	1.148	0.840–1.570	0.387			
Postmenopause (yes vs. no)	1.187	0.866–1.627	0.287			
HPV 16/18 infection (no vs. yes)	0.970	0.687–1.369	0.862			
Histological subtypes (non-SCC vs. SCC)	1.766	1.261–2.473	0.001	1.983	1.401–2.808	<0.001
Tumor size (>4 cm vs. ≤4 cm)	1.521	1.103–2.097	0.010	1.175	0.847–1.631	0.334
FIGO stage (IIA vs. IB)	1.779	1.293–2.448	<0.001	1.307	0.935–1.827	0.117
Node status (yes vs. no)	3.569	2.615–4.870	<0.001	1.881	1.293–2.737	0.001
Depth of myometrial invasion (whole thickness vs. >1/2 vs. ≤1/2)	2.308	1.842–2.893	<0.001	1.628	1.257–2.108	<0.001
Parametrial involvement (yes vs. no)	4.252	2.809–6.438	<0.001	1.822	1.167–2.844	0.008
LVSI (yes vs. no)	2.835	2.058–3.905	<0.001	1.543	1.056–2.254	0.025
KRAS mutation (yes vs. no)	2.577	1.430–4.642	0.002	2.064	1.125–3.787	0.019

CI, confidence interval; FIGO, International Federation of Gynecology and Obstetrics; HPV, human papillomavirus; HR, hazard ratio; LVSI, lymphovascular space involvement; SCC, squamous cell carcinoma.

G13 mutations than in patients with *KRAS* G12 mutations (25.0% vs. 70.6%); however, due to the limited number of cases (21 cases), this difference was not statistically significant (p=0.153) (**Supplementary Fig. 2**).

DISCUSSION

The features of *KRAS* mutations in lung and colon cancer have become increasing clear, whereas the clinicopathological and prognostic characteristics of *KRAS* mutations in cervical cancer remain inconclusive. There are some uncertainties regarding the predictive and prognostic role of *KRAS* mutations in previous studies due to their relatively small sample sizes. In this study, with a large cohort of 876 patients with cervical cancer, *KRAS* mutations were found to be more associated with non-SCC and a positive HPV 18 infection status. In these specific subtypes of cervical cancer, patients with a *KRAS* mutation have a worse prognosis.

The development of a *KRAS* mutation is a rare event in SCCs of the cervix. According to the COSMIC database (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic; Jun 8, 2017), the prevalence of *KRAS* mutations in SCC is approximately 2%, which was confirmed in our patient cohort (2.2%). In comparison, *KRAS* mutations were predominant in non-squamous cell cervical carcinomas, including AC, ASC, and other uncommon subtypes. Spaans et al. [20] demonstrated that *KRAS* mutations occurred more frequently in AC than in SCC (24% vs. 3%, p<0.001), and Wright et al. [21] indicated that *KRAS* mutations were detected only in AC but not in SCC (17.5% vs. 0%, p=0.010). In our cohort of patients with AC, the *KRAS* mutation rate of was 7.3% (8/110), which is similar to the results observed by Ojesina et al. [22] using whole exome sequencing of 24 patients with AC (2/24, 8%). Regarding neuroendocrine carcinomas, Frumovitz et al. [23] reported that the prevalence of *KRAS* mutations was 14% (6/44), which is lower than observed in this study (4/16, 25%). Due to its small sample size, both studies may have some bias. Thus, the reported prevalence of *KRAS* mutations in non-SCC is highly variable.

In the present study, *KRAS* mutations were detected in 3.4% (30/876) of Chinese patients with cervical carcinoma, which was relatively low compared with data from other studies. According to the COSMIC database, the frequency of *KRAS* mutations in cervical cancer is 5.83% (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic; Jun 8, 2017). As the data above indicated, the distribution of the different histological subtypes accounts for the variance of the mutation rate among the studies [21]. In addition, the disease stage might

also contribute to the low frequency of *KRAS* mutations in our cohort of cervical cancer patients. Wegman et al. [24] reported that *KRAS* mutations were more commonly found in patients with advanced stage disease (FIGO stages III–IV) than in those with early stage disease (FIGO stages I–II) (35.3% vs. 5.6%, respectively, p<0.001); however, all our patients were diagnosed with FIGO stages IB–IIA disease.

In accordance with the result of most studies, we confirmed that the 3-year RFS in patients with *KRAS* mutations was significantly lower than that in patients without *KRAS* mutations in our large patient cohort [18,21,24]. Wegman et al. [24] found that among patients treated with definitive chemoradiation, those harboring mutant *KRAS* had significantly worse recurrence-free survival than those with wild-type *KRAS* (p=0.030). Our cohort of patients underwent surgery-based multimodal treatment, and disease recurrence outside of the pelvis was the primary recurrence pattern. Wegman et al. [24] reported that there was a significant association between *KRAS* mutation and distant metastases but not local recurrence, which is consistent with our previous findings. In clinic, the finding of the association between *KRAS* mutation and worse 3-year RFS suggests that detection of *KRAS* mutation could be used as a prognostic marker. Close follow-up is needed in those patients with *KRAS* mutation for early detection of recurrence. In addition to conventional adjuvant therapy, such as concurrent chemoradiation and systemic chemotherapy, further management might be considered in patients with *KRAS* mutation to prevent from recurrence. Novel therapy is needed to be identified including *KRAS*-targeted therapy.

An association between *KRAS* mutations and HPV infection has not been confirmed in the literature, as Wright et al. [21] did not observe an association between HPV infection and *KRAS* mutations in a cohort of 80 patients with cervical cancer. In our study, we demonstrated that *KRAS* mutations were associated with HPV 18 infection but not HPV 16 infection. Moreover, *KRAS* mutations were a predictor of poor disease-free survival (DFS) only in patients with HPV 18 infection. This result is consistent with the finding that an association between *KRAS* mutations and disease recurrence was only observed in patients with non-SCC but not with SCC. Epidemiological studies have confirmed that HPV 18 infection accounts for majority of cervical ACs. In this study, we found that HPV 18 infection was predominantly present in ACs (19.9%), ASCs (23.5%), and uncommon histological subtypes (8.1%) at significantly higher rates than HPV 16 infection (6.0%, 2.5%, and 0.4%, respectively, p<0.001). Compared to SCC, these specific histologic subtypes present poorer survival. Thus, a combination of *KRAS* mutation detection and HPV genotyping might be useful in identifying patient with poor prognosis for further interventions.

According to the literature, *KRAS* mutations at residues G12 and G13 have different risks of tumor progression in lung cancer and CRC [7-9,25,26]. The mechanism of different *KRAS* mutations on tumor progression has not been completely elucidated. It has been revealed that different amino acid changes result in the involvement of different signaling pathways [27-29]. In this study, patients with mutant *KRAS* at codon 13 are more likely to have a shorter DFS than patients with mutant *KRAS* at codon 12, although this difference was not statistically significant. More cases are required to confirm these results. Functional studies of those *KRAS* mutations warrant further studies, especially for the newly identified mutants in cervical cancer.

There are some limitations in our study. First, eligible patients did not include those with advanced cervical cancer, and the frequency, clinicopathological features and prognostic relevance of *KRAS* mutations were obtained from patients with relatively early stage disease,

which could lead to an incomplete analysis of *KRAS* mutations in cervical cancer. Second, the presence of concurrent mutations may also influence the clinical phenotype and prognostic outcomes. Our other study discovered a subset of cervical cancer patients with concurrent *ERBB2*, *PIK3CA*, and/or *KRAS* mutations (sent to publication). A study of a large patient cohort based on whole-genome sequencing is required to fully analyze oncogenic mutations in cervical cancer. Third, the detection of HPV was just limited in HPV 16 and HPV 18. Finally, because a small group of people died of cervical cancer, overall survival was not analyzed in our study.

In summary, *KRAS* mutations were predominant in non-SCC of the cervix and are associated with HPV 18 infection. These mutations were an independent predictor for disease recurrence in patients with cervical cancer who received surgery-based multimodal treatment. Further intervention might be necessary in patients with *KRAS* mutations because of their increased risk for recurrence and distant metastasis. A combination of *KRAS* mutation detection and HPV genotyping would be useful in identifying patient with poor prognosis for further interventions.

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SUPPLEMENTARY MATERIALS

Supplementary Table 1
The association between *KRAS* mutation and disease recurrence

Click here to view

Supplementary Table 2
Detailed mutation and clinicopathological information

Click here to view

Supplementary Fig. 1
Kaplan-Meier curves of RFS for 601 patients with SCCs.

Click here to view

Supplementary Fig. 2
Kaplan-Meier curves of RFS for patients with *KRAS* G12 and G13 mutations.

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