Relationship between Human Papillomavirus Prevalence and DNA Damage in Cervical Cancer Population in Gansu Province, China

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Keywords
Human papillomaviruses · Cervical cancer · DNA damage response

Abstract
Objectives: The aim of this study was to assess the possible reason of the high incidence and mortality of cervical cancer in Longnan, China. Materials and Methods: 147 and 124 invasive squamous-cell carcinoma (SCC) samples from Longnan and different cities and districts of Gansu province were collected in the present study. All the samples were obtained from patients who underwent biopsies with colposcopy or advanced operations and were evaluated by experienced pathologists. HPV genotypes were examined with a validated HPV subtypes kit. The prevalence of HPV infection in SCC patients of China was analyzed by evidence-based medicine in the published literature. The markers of DNA damage response (DDR) – ATMpSer1981, H2AXp Ser139 (γH2AX), Chk-2pThr68, and p53 – were analyzed by immunohistochemistry. Results: HPV positivity, high-risk and multiple HPV positivity, and HPV58 infection were significantly higher in Longnan. Our results show that the prevalence of HPV infection in SCC patients of Longnan are consistent with the HPV prevalence in China. ATM, γH2AX, and p53 expressions in total and HPV+ samples were also higher in Longnan. Conclusions: HPV-related DDR activation may be one reason for the high incidence and mortality of Longnan cervical cancer.

Introduction

Cervical cancer (CC) is a major fatal malignancy, causing about 265,700 deaths yearly in the world. Nearly 90% of CC deaths occur in developing countries. In China, the CC incidence is high, with 132,300 new cases each year, yielding a rate of 27 per 100,000 women [1]. Epidemiological researches and experimental data give evidence that persistent human papillomavirus (HPV) infection is considered to play an important role in the development of CC [2]. The prevalence of HPV infection and the specific distribution of type varies greatly by ethnicity and geographic region. Evidence indicates that HPV16, 18, 33, and 58 are the most common types in women with CC in China [3].

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The genetic instability is relevant to DNA damage response (DDR) in the evolution of CC [4]. Ataxia telangiectasia mutated (ATM) kinase plays a key role in the coordination of DDR. ATM phosphorylation at serine 1981 (ATMpSer1981) by DNA damage activates ATM, subsequently phosphorylates H2AX at Ser139 (γH2AX), Chk2 at Thr68 (Chk2pThr68), and p53 at Ser15 (p53pSer15), which maintain genome integrity by coordinating cell cycle arrest, apoptosis, and DNA damage repair [5].

Persistent infection of high-risk HPV accounts for almost all CC cases [6]. Genotypes HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 are regarded as high-risk types because they are identified in high-grade squamous intraepithelial lesions and invasive CC tissues [7]. Of all these subtypes, HPV16 and 18 are the most carcinogenic and are associated with 70% of CCs [8]. However, a comprehensive overview is missing which compares the HPV oncogenes from various HPV subtypes. Furthermore, discrepancy of the various HPV subtypes in the process of cervical carcinogenesis is not fully understood at the molecular level.

Longnan of Gansu Province, located in the remote region of Northwest China, is a high-incidence area of CC with CC mortality as high as 39/100,000, ranking first in China [9]. Although our previous study has shown genotypes 16, 58, 52, and 18 are among the predominant HPV and HPV infection increased with cervical lesion in Longnan women, the cause for the high incidence and mortality of CC is still unclear [7]. The objective of this study was to analyze and compare the difference of the prevalence of HPV genotypes and DDR between Longnan and other Gansu squamous-cell carcinoma (SCC) patients and to add new evidence that HPV infection related to genetic instability may be one cause for the high incidence and mortality of CC in Longnan.

Materials and Methods

Study Subjects

Between January 1, 2012, and January 30, 2016, a total of 147 samples of SCC from Longnan patients aged 21–76 years and 124 samples of SCC from different cities and districts patients of Gansu province aged 24–79 years were collected for inclusion in the present study. All the samples were obtained from patients who underwent biopsies with colposcopy or advanced operations. Of the Longnan SCC samples, 105 and 18 were obtained, respectively, at the No. 1 Hospital and Child health Hospital of Longnan City as well as 24 samples from Gansu Provincial Cancer Hospital. The SCC samples from different cities’ and districts’ patients of Gansu province were all obtained at Gansu Provincial Cancer Hospital. HPV genotyping tests and immunohistochemical (IHC) analysis of DDR were performed in the pathology department of the medical school. All samples were formalin-fixed and paraffin-embedded, followed by a blinded central pathology review by at least 2 experienced pathologists in the Department of Pathology at the School of Medicine for sections cut concurrently with the IHC slides to confirm the diagnosis. All morphologic reviews were blinded to the outcome. HPV typing status, DDR IHC status, and all DDR IHC reviews were also blinded to the outcome, HPV typing status, and morphologic diagnosis. SCC was diagnosed according to the standard criteria [10]. This study was approved by the Ethics Committees of the Northwest University for Nationalities prior to its start (XBMU-YX-2012002). The characteristics of participant is shown in online supplementary attachment 1 (see www.karger.com/doi/10.1159/000525975 for all online suppl. material).

HPV Genotyping

An HPV genotyping detection kit (Yaneng Bioscience Co., LTD) was used to detect HPV subtypes. For this kit, polymerase chain reaction was used to amplify the L1 gene in conjunction with reverse dot blot to analyze and detect the HPV subtypes [7, 11]. All detection procedures were carried out according to the protocols provided by the manufacturers [11].

Prevalence of HPV Infection in SCC Patients in China by Literature Analysis

In order to acquire a more accurate estimate of the prevalence of HPV infection in SCC patients of China and analyze if our results – the prevalence for HPV in Longnan and Gansu – are consistent with the HPV prevalence in China, we used evidence-based medicine to analyze the published literature. A literature search was conducted in the PubMed database, using dates of publication from inception to December 2018. The search strategy used the following main search terms in combination: “Uterine Cervical Neoplasms,” “CC,” “Cervix tumor,” “squamous cell carcinoma,” “SCC,” “Human Papilloma Virus,” “HPV infection,” “high-risk HPV infection,” “multiple HPV infection,” “China,” etc. (the specific search strategy in online suppl. attachment 2). A reviewer extracted data from full-text papers, and the following information were recorded: study region, study design, multicenter study, number of SCC patients, age range and mean, sample source, infection rates of HPV, high-risk HPV and low-risk HPV, multiple HPV infection rate.

IHC Analysis of DDR

Immunohistochemical analysis was conducted using an automatic immunostainer (DAKO Autostainer link-48) according to the manufacturer’s instructions. The primary antibodies used were as follows: anti-Ser1981 phosphorylated ATM (#05-740, Mouse Monoclonal Antibody, 1:200; Temecula, CA, USA); anti-Ser139 phosphorylated H2AX (#05-636, Mouse Monoclonal Antibody, 1:200; Millipore, Billerica, MA, USA); anti-Thr68 phosphorylated Chk2 (sc-16297-R, Rabbit polyclonal antibody, 1:200, Santa Cruz Biotechnology, Dallas, TX, USA), anti-p53 (DO-1, sc-126, Mouse Monoclonal Antibody, 1:200, Santa Cruz Biotechnology Inc., Dallas, TX, USA). Cancer cells showing nuclear staining, without regard to the presence of cytoplasmic staining, were considered as positively immunostained for ATM, γH2AX, Chk2, and p53 [12].
Evaluation of IHC Staining

Sections were semiquantitatively scored as follows: (0+): 0% immunoreactive cells; (1+): less than 5% immunoreactive cells; (2+): 5–50% immunoreactive cells; (3+): more than 50% immunoreactive cells. At last, for statistical purposes and to define a cutoff level, slides with 0 and 1 scores were defined negative, and those with 2 and 3 scores were considered positive [13].

Statistics

Statistical analysis was performed using SPSS version 21.0 (SPSS, Chicago, IL, USA). Differences between rates were tested by the χ² test and Fisher’s exact tests according to the characteristics of the data distribution at a 5% significance level. The significance level α was set at 0.05.

Results

Genotypes Detected in SCC

All the 147 Longnan and 124 Gansu SCC samples were analyzed to detect and characterize HPV infection. The HPV positive rate was 90.00% (132/147) in Longnan samples and 75.81% (94/124) in Gansu samples; HPV positivity was significantly higher in Longnan than that in Gansu (p < 0.01, Table 1). The high-risk HPV positivity of Longnan was also higher than Gansu (100%, 132/132 vs. 95.75%, 90/94, p < 0.05, Table 1).

Some samples were infected with more than 2 types of HPV simultaneously. Compared to Gansu samples, more patients of Longnan had multiple HPV infections (24.24%, 32/132 vs. 11.70%, 11/94, p < 0.05, Table 1). Double infections accounted for the majority of multiple infections. Of those, HPV16 plus other types were the most common combinations in both Longnan and Gansu samples.

The distribution of HPV genotypes is shown in Figure 1. HPV16 was the most common genotype in SCC of Longnan samples, accounting for 76.52% (101/132), followed by HPV58 (20.46%, 27/132) and HPV18 (6.82%, 9/132). For Gansu samples, HPV16 was also the most common genotype, accounting for 73.40% (69/94), fol-
lowed by HPV18 (13.83%, 13/94) and HPV58 (6.38%, 6/94). As shown in Figure 1, no differences in the genotypes detected in the two locations were significant except HPV58 infection in Longnan compared to Gansu samples ($p < 0.01$).

**Prevalence of HPV, High-/Low-Risk HPV, and Multiple HPV in Chinese SCC Patients**

As shown in Table 2, 14 other article-related SCC patients in China and HPV infection were included in our study [14–26]. Among them, 5 studies reported a >90% infection rate of HPV in SCC patients [14, 17, 19, 22, 26], and the HPV positive rate was all more than 80% in other studies except one study [15]. We also analyzed the high-risk HPV and low-risk HPV positivity in these studies. To learn a more accurate estimate of the prevalence of some susceptible to infection HPV types in SCC patients in China, we also analyzed the HPV-positive rate of HPV16, 18, and 58. The result displayed that the highest HPV16-positive rate was 89% in Linzhou city [22]. In addition, the rates of multiple HPV infections have big difference in included researches (3.4–40%).

**Activation of DDR in SCC Samples**

In total, 100 Longnan and 81 Gansu SCC samples were used to analyze the markers of DDR activation (Fig. 2). In the present study, part of the SCC tissues showed positive staining for ATM, as well as γH2AX, Chk2, and p53 in the nucleus. The results of ATM, γH2AX, Chk2, and p53 testing in Longnan and Gansu samples are given in Table 3. With the exception of Chk2, the expressions of ATM, γH2AX, and p53 were significantly higher in samples from Longnan compared to the rest of Gansu ($p < 0.01$, Table 3).

For simple infections, the results of ATM, γH2AX, Chk2, and p53 in Longnan and Gansu samples are given in Table 3. There was a significant difference in the expression of ATM and γH2AX between Longnan and Gansu samples ($p < 0.05$, Table 3). For multiple infections however there was no difference in the activation of DDR between Longnan and Gansu samples ($p > 0.05$, Table 3).

**Correlation between HPV Genotypes and DDR Activation in Longnan and Gansu SCC Specimens**

We divided the samples into two HPV+ and HPV− parts to compare the activation of DDR between Longnan and Gansu samples. Table 4 details the results for the activity of ATM, γH2AX, Chk2, and p53 in HPV+ samples from Longnan and Gansu. Except for Chk2, the expression of ATM, γH2AX, and p53 were also significantly higher in samples from Longnan than Gansu ($p < 0.05$, Table 4). For HPV− samples, no difference was shown for the activation of DDR between Longnan and Gansu samples ($p > 0.05$, Table 4).

Lastly, we compared the DDR activation in HPV16+, HPV18+, and HPV58+ samples between Longnan and Gansu. The results of ATM, γH2AX, Chk2, and p53 testing in HPV16+ samples from Longnan and Gansu are shown in Table 5. The expression of γH2AX and p53 in Longnan samples was significantly higher than in Gansu.
Table 2. Published study findings on the prevalence of HPV types and high-risk or low-risk HPV infection, multiple HPV infections of SCC patients in China

| Source            | Region             | Study design     | Multi-center study | SCC patients, n | Age range (mean) | Sample source          | HPV positive, n/N (%) | HR-HPV positive, n/N (%) | LR-HPV positive, n/N (%) | HPV16 positive, n (%) | HPV18 positive, n (%) | HPV58 positive, n (%) | Multiple HPV infections positive, n (%) |
|-------------------|--------------------|------------------|-------------------|-----------------|-----------------|------------------------|-----------------------|------------------------|------------------------|------------------|------------------|------------------|--------------------------------------|
| Wang 2018 [14]    | Southeast China    | Retrospective    | Yes               | 326             | 18–78 (43.6)    | Cervical swab          | 318/326 (97.5)        | NR                     | NR                     | 73/326 (22.3)     | 115/326 (35.2) | 54/326 (16.5) | 64/326 (19.63)                        |
| Liao 2019 [15]    | Ganzhou city       | Retrospective    | No                | 108             | 29–81 (48.8)    | Tissue blocks          | 69/108 (64.3)         | 82/108 (75.9)         | 3/108 (2.77)          | 5/108 (50)        | 10/108 (9.3)   | 6/108 (4.7)    | 14/108 (13.2)                          |
| Li 2019 [16]      | Beijing city       | Retrospective    | No                | 76              | 18–74 (41.2)    | Cervical swab          | 67/76 (88.4)          | 62/76 (82.1)          | NR                     | 55/76 (72.4)     | 9/76 (1.2)     | 5/76 (6.6)     | 30/76 (39.47)                          |
| Chen 2018 [17]    | China              | Retrospective    | Yes               | 630             | NR (45.6)       | Tissue blocks          | 615/630 (97.6)        | 605/630 (98.4)        | 11/615 (1.8)          | 483/615 (78.5)   | 49/615 (8.0)   | 14/615 (2.3)  | 21/615 (3.4)                           |
| Yuanyue 2018 [18] | Yunnan province    | Retrospective    | No                | 68              | 21–86           | Biopsy sample         | 60/68 (88.2)          | 56/68 (82.3)          | 11/68 (16.2)          | 20/68 (29.4)     | 8/68 (11.8)    | 10/68 (14.7) | 14/68 (20.6)                          |
| Zhang 2017 [19]   | Eastern China      | Retrospective    | Yes               | 440             | NR              | Cervical swab         | 416/440 (94.5)        | 370/440 (84.1)       | NR                     | 212/440 (48.2)   | 20/440 (4.5)   | 36/440 (8.2)  | 68/440 (15.45)                         |
| Zhao 2017 [7]     | Longnan city       | Retrospective    | No                | 137             | 17–79 (43.5)    | Biopsy sample         | 122/137 (89.05)       | NR                     | NR                     | 96/122 (78.69)   | 8/122 (6.56)   | 25/122 (20.49) | 31/122 (25.41)                        |
| Li 2016 [20]      | Shaanxi province   | Case-control     | No                | 1,747           | 26–69 (45.67)   | Cervical swab         | NR                    | 1,150/1,747 (65.82)  | 0/1,747 (0)         | 576/1,747 (32.97) | 100/1,747 (5.72) | 111/1,747 (6.33) | 363/1,747 (36.18)    |
| Xiao 2016 [21]    | Beijing city       | Retrospective    | Yes               | 766             | 19–79 (43.5)    | Biopsy sample         | NR                    | (752/766) (98.2)     | NR                     | 556/752 (74)     | 54/752 (7.2)   | 179/752 (23.8) | 244/752 (32.44)                        |
| Liu 2014 [22]     | Linzhou city       | Retrospective    | No                | 75              | NR (47)         | Biopsy sample         | 70/75 (93.3)          | 66/75 (88.0)          | 4/75 (5)              | 62/75 (89)       | 7/75 (10)       | 11/75 (16)     | 5/75 (7)                              |
| Abudukadeer 2010 [23] | Xinjiang province | Retrospective    | Yes               | 91              | 18–78 (48)      | Tissue blocks         | 80/91 (88)            | NR                     | NR                     | 77/91 (85)       | 14/91 (15)      | 2/91 (2)       | NR                                   |
| Wu 2008 [24]      | Sichuan province   | Retrospective    | No                | 190             | 17–71 (46)      | Biopsy sample         | 159/190 (83.7)        | 101/190 (53.2)        | NR                     | 111/190 (58.4)   | 14/190 (7.4)   | 13/190 (6.8)  | 76/190 (40)                           |
| Liu 2008 [25]     | Hong Kong          | Case-control     | Yes               | 96              | 23–89 (55.8)    | Cervical swab         | 96/96 (89.6)          | 86/96 (94.6)          | 2/96 (2.1)           | 44/96 (45.8)     | 13/96 (13.5)  | 8/96 (8.3)    | 5/96 (5.21)                           |
| Wu 2006 [26]      | Nanchang/ Zhumai city | Case-control     | Yes               | 223             | 22–84 (46.7)    | Biopsy sample         | 207/223 (92.8)        | NR                     | NR                     | 157/223 (70.4)   | NR       | NR           | NR                                   |

SCC, squamous-cell carcinoma; NR, not reported; HR-HPV, high-risk HPV, includes HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59; LR-HPV, low-risk HPV, includes HPV genotypes 6, 11, 42, 43, and 44.
samples ($p < 0.05$, Table 5). For HPV18+ samples, the results of ATM, γH2AX, Chk2, and p53 testing in Longnan and Gansu are listed in Table 5. The only significant difference seen was in the expression of Chk2 between Longnan and Gansu ($p < 0.05$, Table 5). For HPV58+ samples, the results of ATM, γH2AX, Chk2, and p53 testing in Longnan and Gansu are shown in Table 5. However, no difference of DDR activation was shown between Longnan and Gansu samples ($p > 0.05$, Table 5).

### Discussion

In the current study, the distribution of HPV genotypes among women with SCC in Longnan and Gansu were identified. First, the data confirm the higher positive rate of HPV DNA in Longnan patients. The prevalence of HPV infection for all types in CC varies widely [7, 14, 16–19, 22–26]. Our results – 89.80% and 75.81% in specimens positive for HPV in Longnan and Gansu, respectively – are consistent with most others. Second, our find-
ings confirm that high-risk HPV infection in Longnan samples is also higher than in other parts of Gansu (100% vs. 95.75%). All the HPV genotypes detected in Longnan samples are high-risk types. Chen [17] reported the highest high-risk HPV-positive rate (98.4%) in China, and Yuanyue [18] reported the highest low-risk HPV-positive rate (16.2%) in Yunnan province. The third important issue in this study is that the prevalence of multiple HPV infections in Longnan patients is higher than in other Gansu samples (24.24% vs. 11.70%). The reported incidence of multiple HPV infection in SCC varied greatly and ranges between 3.4 and 40% in China [14–25]. There was research showed that patients infected with multiple HPV genotypes had a 31.8-fold higher risk of CC, while those infected with a single HPV genotype only had a 19.0-fold increased risk [27]. Last, the distribution of dominant HPV genotypes showed obvious differences between Longnan and Gansu samples. In the present study, HPV16 was the dominant genotype (73.40% vs. 76.52%), and HPV18 (6.82% vs. 13.83%) and 58 (20.46% vs. 6.38%) were the second most dominant genotypes. HPV58 infection in Longnan is statistically higher than in Gansu. HPV16 infection in our study is consistent with most reports [28–30]. HPV18 is another one of the most carcinogenic HPV genotypes which accounting for 10~15% of CCs [28, 30]. The prevalence of HPV18 in Longnan in our result is a little lower but consistent with some reports in China [19, 20]. Although the overall prevalence of HPV58 in Longnan is much higher than the reports from Japan and South Korea (in which HPV58 prevalence are between 3.3%~19% in patients with SCC) [31], our resulting HPV infection prevalence in Longnan and Gansu are consistent with the prevalence in China [14–25].

Then our research provides evidence that ATM is activated in SCC specimens, and this pattern of ATM activation suggests an intriguing possibility that ATM plays an important role in tumor surveillance. The concept of ATM involvement in tumor surveillance is supported by other publications which proved an increase in ATM activation in lung and bladder precancerous lesions and decreased function of ATM in prostate tumorigenesis [32]. Consistent with these studies, we also proved the expression of Chk2, γH2AX, and p53 in CC. This activation pattern of Chk2, γH2AX, and p53 is consistent with their involvement in the surveillance of cervical tumorigenesis via ATM. Most importantly, in this study, we found significantly enhanced expression of ATM, γH2AX, and p53 in Longnan specimens compared to Gansu. This finding demonstrated that DNA damage checkpoints not only become activated but also undergo stronger activation in the process of cervical tumorigenesis in Longnan patients.

We also evaluated simple and multiple infections and the various HPV subtype induced DDR in cervical carcinogenesis. First, the differences of cervical carcinogenesis between simple and multiple infections were compared between Longnan and Gansu. No statistically significant difference was shown for the expression of DDR in the multiple infections; however, for simple infection, ATM

|                | HPV16 + CC† Pearson’s χ² test | p value | HPV18 + CC‡ p value | HPV58 + CC‡ p value |
|----------------|-------------------------------|---------|---------------------|---------------------|
|                | ATM                           |         | γH2AX               | Chk2               | p53                 |
|                | Negative                      |         | Negative            | Negative            | Negative            |
| Gansu          | 31                            | 1.559   | 0.212               | 0.396               | 0.254               |
| Longnan        | 35                            |         | 4                   | 1                   |                     |
|                | Positive                      |         | Positive            | Positive            |                     |
| Gansu          | 15                            | 4.000   | 0.000               | 0.119               | 0.535               |
| Longnan        | 28                            |         | 11                  | 4                   |                     |
|                | Chk2                          |         | Chk2                | p53                |                     |
| Gansu          | 35                            | 0.131   | 0.717               | 0.046               | 1.000               |
| Longnan        | 46                            |         | 11                  | 1                   |                     |
|                | p53                           |         | p53                 | p53                |                     |
| Gansu          | 42                            | 4.220   | 0.040               | 0.063               | 0.470               |
| Longnan        | 48                            |         | 12                  | 3                   |                     |
|                | p53                           |         |                     |                     |                     |

† Pearson’s χ² test for significance for HPV16+ CC. ‡ Fisher’s exact test for significance for HPV18+ and HPV58+ CC.
and γH2AX activation was statistically higher in Longnan specimens. This pattern of DDR activation further proves that earlier oncogenic events result in ATM activation and imply ATM plays a role in tumor surveillance in Longnan patients. Owing to our relatively small sample size of multiple infections, we may not be able to exclude the possibility that ATM also activates Chk2, γH2AX, and p53.

We then compared the differences of cervical carcinogenesis in HPV+, HPV16+, HPV18+, and HPV58+ groups between Longnan and Gansu patients. The fact that ATM, γH2AX, and p53 activation is statistically higher in Longnan for HPV+ groups suggests that genetic instability is much more serious in Longnan patients. However, as ATM activation is not statistically higher in Longnan for HPV16+ and HPV18+ specimens, Chk2, γH2AX, and p53 remain elevated in the above positive groups which may be attributable to (1) the possible longer half-life of Chk2, γH2AX, and p53 than the half-life of activated ATM or (2) additional signals other than those of tumor surveillance in cervical lesion tissue resulting in activation of Chk2, γH2AX, and p53 in Longnan patients. The relatively small size of HPV58+ samples may be one reason that no difference was shown between Longnan and Gansu.

Although our present study supplied new data that the distribution of HPV genotypes and DDR related to the high incidence and mortality of CC in Longnan patients, there are some limitations in our study. First, the sample size of this study is relatively small. We only enrolled a total of 271 samples for HPV genotypes and 181 samples for DDR markers study in the present research. As a result, there are only 5 samples of HPV− in Longnan and 4 samples of HPV58+ in Gansu were included in this study. In addition, the various ethnic and socio-economic backgrounds related to the incidence and mortality of CC were not fully taken into account in present study. Although CC mortality has shown a decreased trend in most provinces in recent decades in China, in some regions of the western regions, where the economic situation is poor, the rate of CC mortality has not changed significantly. Some areas, such as Longnan of Gansu province, the mortality rate for CC is even among the highest in the world. The reason for the high mortality rate of CC in Longnan is that the area is located in a region with low socio-economic levels in the western parts of China (Longnan is a national poverty county). The local transportation is inconvenient, medical and health facilities are not perfect, and the level of diagnosis and treatment is limited in this area. Another reason is the uses of Pap smear for CC screening in this area is less than in the city. At last, the increase of high-risk HPV infection in this area, which may be caused by multiple sexual partners, women’s cervical columnar epithelium, urinary tract infection, and abnormal vaginal microbiota, may be one reason of the high mortality rate of CC [33].

**Conclusion**

In summary, the results of the present study add to the evidence that HPV results in alterations on the genetic level and triggers the cervical carcinogenic process. The distribution of HPV genotypes and related DDR may be one reason for the high incidence and mortality of CC in Longnan patients.

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**Statement of Ethics**

This study protocol was reviewed and approved by the Ethics Committees of the Northwest Minzu University, approval number (XBMU-YX-2012002). The trial was registered in the Chinese Clinical Trial Registry http://www.chictr.org.cn; registration No.: ChiCTR-TRC-1800016405; principal investigator: Zhong Guo; date of registration: 31 May 2018. Written consent has been obtained from each patient to participate in this study.

**Conflict of Interest Statement**

The authors have no conflicts of interest to declare.

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Author Contributions

This study was conceived and designed by Zhao Jin, Zhou Yunsong, and Guo Zhong. Bioinformatic analysis was performed by Zhou Yunsong, Qu Hongmei, and Zhu Tianyuan. The manuscript was drafted by Zhao Jin and edited by Wang Chenjing, Si Tianbin, and Wang Qiang.

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Data Availability Statement

The data that support the findings of this study are not publicly available due to possible patent application, and further inquiries can be directed to the corresponding author.