RESEARCH ARTICLE

Integration Sites and Genotype Distributions of Human Papillomavirus in Cervical Intraepithelial Neoplasia

Li Wang[1], Shu-Zhen Dai[2]*, Hui-Jun Chu[2], Hong-Fei Cui[1], Xiao-Yan Xu[1]

Abstract

Objectives: To analyse HPV integration prevalence and genotype distributions in cervical intraepithelial neoplasia (CIN) in east part of China, furthermore to assess preferential sites for common HPV integrations and provide baseline information for cervical abnormality screening and prevention. Methods: Integration of HPV in 113 paraffin-embedded cervical intraepithelial neoplasia samples was assessed using Gencap technology in Key Laboratory of Biotechnologies in BGI-Shenzhen. Results: 64 samples were HPV-integrated and as the cervical lesions increased, the integration rate became higher significantly (*P*=0.002). Fifteen different HPV genotypes were detected, 14 high-risk (16, 18, 31, 33, 51, 52, 56, 58, 66, 68) and 1 low-risk (11). The most common genotypes were HPV-16, 58, 33, 52, 66, and 56. Thirteen patients had co-integration involving mainly HPV-16 and 58. The frequency of HPV gene disruption was higher in L1 and E1 genes than in other regions of the viral genomes. Conclusion: Some 56.6% of CIN lesions in Qingdao had HPV integrations, and 67.2% of HPV-integrated patients were HPV-16 and 58, more prone to be integrated in younger patients below 45 years old. There exist preferential sites for HPV-16 and HPV-58 integration, and they are more likely to be disrupted in the L1 and E1 loci.

Keywords: Cervical intraepithelial neoplasia - HPV - integration - HPV genotype - preferential sites

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Introduction

Cervical cancer is the second most frequent cancer and a leading cause of mortality worldwide (Parkin et al., 2005). Persistent infection of Human Papillomavirus (HPV) is necessary for the development of cervical intraepithelial neoplasia and invasive cancer (Bosch et al., 2007). There is a unique relationship between HPV and cervical cancer while no other major human cancer is dependent on a single factor for its development (Walboomers et al., 1999; Bosch et al., 2002). As the epidemiological studies had provide all evidence that the infection of Human Papillomavirous is very common in young women who have sexually active, the prevalence can reach as high as 76–80% (Brown, 2005) while the worldwide HPV prevalence in cervical caner was estimated between 85% and 99% (Coutlee et al., 2011). More than 120 genotypes of HPVs isolated from human in current classification, and at least 18 high-risk HPV genotypes have been identified in human genital ducts. High-risk HPV genotypes are believed to increase the risk of cervical precancerous lesions and trigger the progression of these lesions to carcinoma, the most prevalent ones worldwide and in Asia are HPV-16 and HPV-18 (Smith et al., 2007; Quek et al., 2013). The etiologic role of most common high-risk HPV types such as HPV-16 and 18 in cervical abnormalities has been demonstrated by molecular studies, but the integrations of each types of HPV has rarely been reported. Over the past twenty years, two new preventive approaches have emerged, first the vaccination for the primary prevention of HPV-16 and 18 infections in adolescent girls, second is the detection of infections with carcinogenic HPVs that allow secondary prevention via the identification and treatment of precancerous lesions and early-stage cervical cancers (Wheeler et al., 2009).

Although most women are infected with high-risk human papillomavirus at some time, few will progressed to invasive disease (Cricca, 2009), because most HPV infections could regress within two years and only a minority of women will develop persistent HPV infection that could eventually cause cervical intraepithelial neoplasia (CIN) (Ramanakumar et al., 2010). The integration of HPV DNA into the human gene is thought to occur early in cancer development and to be an important event in malignant transformation of cervical cancer (Ho et al., 2011). Integration of human papillomavirus into the host genome has been proposed as a potential marker of cervical neoplastic progression. The reports about prevalence of HPV infections of cervical abnormalities were common in the world, but integrations and genotypes' distribution was rarely reported. Therefore,

*[1]Qingdao University Medical College, [2]Key Laboratory of Cervical Disease of Qingdao, Department of Obstetrics and Gynecology, Affiliated Hospital of Qingdao University Medical College, Qingdao University, Qingdao, China *For correspondence: wlrst@126.com*

Table 1. The Integrations of Different HPV Genotypes

HPV genotypes	classification	number of patients	Prevalence (%)
single integration			
HPV-16	HR	26	40.6
HPV-18	HR	1	1.56
HPV-31	HR	1	1.56
HPV-33	HR	4	6.25
HPV-51	HR	1	1.56
HPV-52	HR	3	4.69
HPV-56	HR	2	3.13
HPV-58	HR	9	14.1
HPV-66	HR	3	4.69
HPV-68	HR	1	1.56
co-integrations			
HPV-11/33	LR/HR	1	1.56
HPV-16/33	HR/HR	2	3.13
HPV-16/52	HR/HR	1	1.56
HPV-16/58	HR/HR	4	6.25
HPV-39/66	HR/HR	1	1.56
HPV-16/45/66	HR/HR/HR	1	1.56
HPV-52/53/58	HR/HR/HR	1	1.56
HPV-31/51/58	HR/HR/HR	1	1.56
HPV-56/58/59/68	HR/HR/HR/HR/HR	1	1.56

the aim of our study was to examine the prevalence of HPV integrations and genotypes of HPV in cervical intraepithelial neoplasia in Qingdao, a big east city in China, in order to provide some baseline information for future screening and prevention programs.

Materials and Methods

Ethical consideration

The research was approved by the Committee for Ethics in Research Involving Humans at the Qingdao University.

Collections of clinical specimens

113 cervical paraffin-embedded samples were collected from the Department of Obstetrics and Gynecology in Affiliated Hospital of Qingdao University. All patients were selected from May 2009 to October 2011, based on the criteria: all patients had a colposcopy because of an abnormal pap smear or a positive HPV test, the pathology of biopsy were cervical intraepithelial neoplasia, and then gave them the cold-knife conization or leep , the final pathology were unanimous with the former.

Methods

DNA extraction was carried out using Magen DNA FFPE Tissue kit by the PerkinElmer company, and then tested by the Nanodrop 1000 (Thermo Fisher Scientific Company). HPV genotyping was performed by using the MY09/MY11 primers which amplifying a fragment of 190 bp in the L1 gene. HPV-16 and 58 positive samples were chosen to do the integration analysis, 20μg of samples was transferred to a 100 μl in a microtube (6*16 mm) which was discarded in the Covaris S2 (Covaris Company,Woburn, Massachusetts, USA), the DNA was divided into 200bp to 300bp fragments. We have an Cooperation with Big Data & Gigascience Company and the following steps were finished in Shenzhen Key Laboratory of Biotechnologies, (BGI-Shenzhen, Shenzhen, China, Asia) using the MyGenostics GenCap Technology.

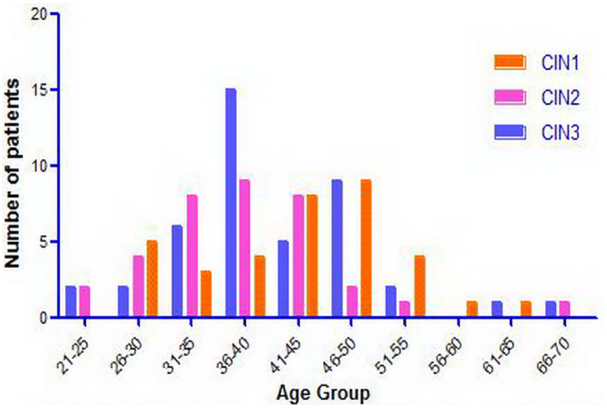

Figure 1. The Distribution of Cervical Intraepithelial Neoplasm Patients by 5-year Age Group in 113 Patients

Statistical analysis

The results were analyses using the Sigmastat package software (IBM, SPSS Inc) and Graphpad Prism 5 (Graphpad Software, La Jolla, CA, USA), the statistical significance was set at $P<0.05$.

Results

Clinical data

The age of the 113 patients raged between 23 to 69 years, the mean age was 40.64±8.982 years. By the pathology of these cervical samples, CIN 1 patients were 35 (31%), CIN 2 patients were 35 (31%) and CIN 3 patients were 43 (38%). The distribution of these 3 histopathological types by 5-year age groups was showed in Figure 1.

Integration of HPV and genotyping

Of all the 113 cervical intraepithelial neoplasia samples, 64 (56.64%) samples were detected HPV integrations, integrations in CIN1, CIN2 and CIN3 were 13, 18 and 33 samples, account for 20.31%, 28.13% and 51.56% respectively. HPV integration rate in CIN1 was 37.14%, CIN2 was 51.43% and CIN3 was 76.74% (P=0.002). In total, 15 types of HPV were detected, 14 of them (16, 18, 31, 33, 51, 52, 56, 58, 66, 68) were commonly considered as high-risk papillomavirus while 1 (11) was classified as low-risk HPV. Among all these samples, the prevalence of each HPV integrations in cervical intraepithelial neoplasia patients is listed in Table.1. The results showed that the most common HPV genotypes with single integration were HPV-16 (40.6% of the integrated-patients), HPV-58 (14.06%), HPV-33 (6.25%), HPV-52 (4.69%), HPV-66 (4.69%), and HPV-56 (3.13%). The HPV-18, 31, 51, 68 had only integrated in one patient each. The co-integrations of HPV in CIN patients were also listed in Table.1. HPV-16/58 accounted for 6.25% (4/64), HPV-16/33 accounted for 3.13% (2/64), while other co-integrations as HPV-11/33, HPV-39/66, HPV-16/52, HPV-31/51/58, HPV-52/53/58, HPV-16/45/66, and HPV-56/58/59/68 were accounted

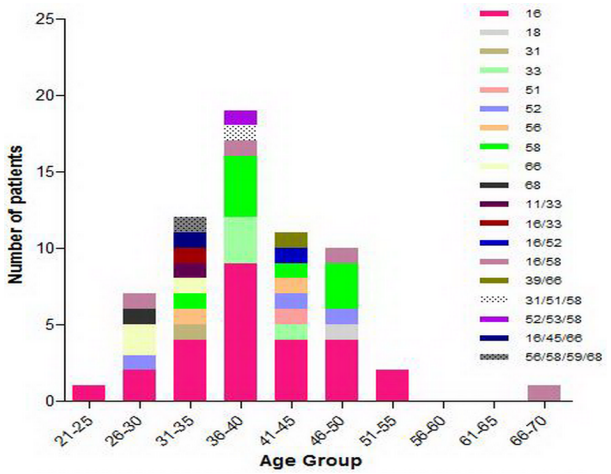

Figure 2. The Distribution of HPV Genotypes by 5-year Group in 64 HPV Integration Patients

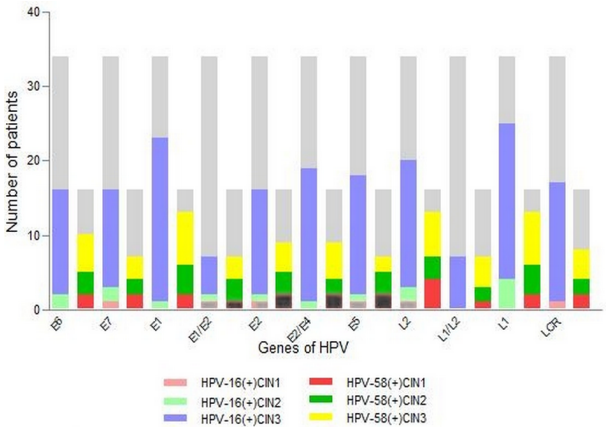

Figure 3. Vitual Diruption Sites of HPV-16 and HPV-58 in the Different Grades of CIN Patients

1.56% each. Whether co-integrations were taken into consideration or not, HPV-16 and HPV-58 were the most common two genotypes, they affected 43 patients, 67.2% of all the integrated patients, and were more frequent in younger group ≤45 years than in elder patients (47% and 20% respectively, $P<0.05$). The age of HPV integrations distribution were shown in Figure 2. The results showed that between 31 and 50, the CIN patients have high integration rate, it accounts about 81.25% (52/64) of all the integrated patients. When each genotype was taken into consideration, HPV-16 integrated patients in this ages were 21, accounted 80.77% in all the HPV-16 integration patients. HPV-58 and HPV-33 integrated account 100% at the age 31 to 50.

Distribution sites in the HPV-16 and HPV-58 genomes

As HPV-16 and HPV-58 have the most frequently integrations, the sequence analysis was made to show that sites of viral gene disruption occurred from E6 to L1 genes. Among the 34 HPV-16 integration and 16 HPV-58 integration samples, distributions were more frequently in the L1 gene which account for 73.52% and 81.25% respectively. Then followed by E1 gene, it was 67.64% and 81.25% of each sample. Furthermore, CIN2 and CIN3 samples showed disruptions in a higher rate of HPV L1 and E1 gene, demonstrating their association with higher grade of cervical lesions (Figure 3).

Discussion

Epidemiologic and molecular biologic studies have shown that the most important etiologic risk factor for the development of cervical cancer is the high-risk (HR-HPV) HPV infection, and the integration of HR-HPV is considered to be a key event in the progression of CIN to invasive cancer (Doorbar, 2007).

Elucidating these particular integration events is important to understand HPV-induced carcinogenesis (Li et al., 2013). There are reports about viral genome integration occurs during the progression of cervical preneoplasms to invasive cancers at one or several cell chromosomal loci (Nogara et al., 2012). In our study, we chose 113 paraffin-embedded samples which histopathology were CIN1 to CIN3, 64 (56.64%) of them were detected HPV integration, the rate of HPV integration among different grades of CIN showed a significantly difference, indicated that HPV integration distinguish women with a grater propensity for lesion progressions.

Irrespective of cervical cytology, HPV16 was the most prevalent genotype detected in the world (Clifford et al., 2005). From a large Chinese meta-analysis study, the most frequent types were HPV16, 58, 52, 18, 39, 33, 68, 31, 66 and 6 in women with normal diagnoses (Bao et al., 2008). Based on our research of HPV integrations in cervical intraepithelial neoplasia of Qingdao, 15 genotypes of HPV were detected, 14 of them (16, 18, 31, 33, 51, 52, 56, 58, 66, 68) were commonly considered as high-risk papillomavirus while 1 (11) was classified as low-risk HPV. HPV-16 and HPV-58 were the most frequently integrated, some researchers reported that HPV-58 and 52 are relatively prevalent genotypes in Asian including Chinese women (Ochi et al., 2008). Furthermore, HPV-16 and HPV-58 was not only have a high frequent in single infection but also in the multi-infections. This result was similar to Shen et al. (2013) did the study in Henan Province in the middle area of China, and so did the study by Jinke Li et al. (2011) in Chengdu, the western area of China.

HPV screening and vaccination is an effective measure in cervical lesions prevention, which is widely used in many infectious diseases (McCredie et al., 2008; Schiffman et al., 2008). Vaccines were developed against HPV infection to prevent cervical cancer and other HPV related diseases, so the availability of HPV vaccines will help to not only curb the incidence and mortality of cervical cancer, but also reduce the cost burden of cervical cancer screening programs (Li et al., 2011). Gardasil® is a bivalent vaccine that protect against HPV-16 and 18, it is being widely introduced in Western countries and new broad-spectrum HPV vaccines are in development. Our result showed that 56.64% of CIN patients are integrated HPV of which 67.2% (43/64) are HPV-16 and HPV-58 that are not covered by the available vaccines. To our contrast, HPV-18 was detected in only 1.6% and 0.9% of the HPV-integration patients and all the CIN patients.

The patients of CIN who have HPV integration were most at the age of 31 to 50 years, each types of HPV are more likely to concentrate around 36 to 40 years old. At this age, the host immune system often trend to calm down,

clearance of viral infections was influenced by viral-host interactions (Wang et al., 2003). In former studies, most researchers paid attention only on the rate of integrations in cervical abnormalities. In our study, we correlated the clinical and experimental data, we found that older patients had lower HPV integration rates than did younger patients, which is consistent with a previous report suggesting that high-risk HPV may be more oncogenic in younger women (Porras et al., 2009). Younger patients with cervical intraepithelial neoplasia may need a more regularly follow-up.

In our study, we cooperated with BGI Company (Shenzhen, China) and used the GeneCap technology which can detect integration sites more effectively. The results show distributions of HPV-16, 58 were more frequently in the L1 and E1 gene in cervical intraepithelial neoplasia, this differed from some previous report (Li et al., 2013). There may exists some reasons for this: first, HPV integration into the host genomes does not appear to be an entirely random event but occurs preferentially at certain chromosomal locations, HPV genomes could be disrupted at any gene, cells with viral disruption at the L1 genes may be selected against during the clone selection process (Li et al., 2013). Second, most previous studies chose the cervical cancer samples, but we use the cervical lesions at an early stage, their cells may contain different cell clones. The disruption of HPV E1 gene was more frequently detected in high grade of CIN, which was not consistent with the former reports that suggested E2 have a higher integration rates. Integration usually disrupts the E1 or E2 genes, potentially leading to a deregulation of viral gene expression. The E1 and E2 proteins are important for transcriptional regulation, replication and segregation of viral DNA, one of E1's functions is to initiate DNA replication of DNA, but the exact role of E1 protein is not well characterized. The disruption of the E2 repressor allows over-expression of the E6 and E7 oncoproteins, which might promote the development of neoplasia, E1 gene integration may happened at the early stage of cervical lesion development. Our data revealed L1 and E1 were the most frequently integrated into the human genomes. Furthermore, CIN2 and CIN3 samples showed disruptions in a higher rate of HPV L1 and E1 gene, demonstrating their association with higher grade of cervical lesions, indicates that HPV L1 and E1 integration may distinguish women with a propensity for lesion progression.

In conclusion, 56.63% cervical intraepithelial neoplasia patients in our study are associated with HPV integration. HPV integration and distributions may promote cervical lesions progression. The two most common HPV genotypes were HPV-16 and 58 which formed 67.2% of HPV integrations and 38.05% of all CIN patients. Patients below the age of 45 years old have a higher HPV integration rate than the older patients. HPV-16 and HPV-58 integration and disruption are not entirely random events but occur at preferred sites. These data may provide important information for HPV detection and genotyping in cervical abnormalities that can guide future applications of screening and prevention measures in China.

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