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ORIGINAL ARTICLE

Comprehensive Data of *P53* R282 Gene Mutation with Human Papillomaviruses (HPV)-Associated Oral Squamous Cell Carcinoma (OSCC)

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Abstract

Alterations of the *P53* gene and human papillomavirus (HPV) infection are associated with development of oral squamous cell carcinoma (OSCC). We aimed to identify mutation of *P53* exon 8 codon 282 in OSCC and correlate these with HPV infection as well as histopathological grade of OSCC. Samples of known HPV infection status were studied including oral lesion cells, formalin-fixed paraffin embedded (FFPE) tissues from OSCC and exfoliated oral cells of matched age-sex controls. *P53* exon 8 mutation was detected using the polymerase chain reaction (PCR). Mutation of codon 282 was identified by allele-specific oligonucleotide typing (ASO) using EvaGreen real-time PCR. The PCR products were analyzed by gel electrophoresis and melting curve analysis. Mutation of *P53* exon 8 was seen in 81.7% and 69.6% of FFPE OSCC tissues and oral lesion cells, respectively. This was significantly higher than in controls (16.7%). Frequency of mutation did not differ between HPV-positive samples (62.5% and 81.8% in oral lesion cells and FFPE tissue samples, respectively) and HPV-negative samples (73.3% and 81.5% in oral lesion cells and FFPE tissue samples, respectively). This finding is similar to *P53* codon 282 mutation that was found only in FFPE tissues (35.0%) and oral lesion cells (32.6%) from both HPV-positive and negative OSCC. Interestingly, frequency of mutation was higher in well-differentiated OSCC with HPV-infection (28.1%) than without HPV (14.8%). This result demonstrated a mutation hot spot in *P53* associated with oral carcinogenesis and might be useful to guide chemotherapeutic modality for HPV-associated OSCC in northeast Thailand.

Keywords Human papillomavirus · HPV · *P53* mutation · *P53* exon 8 codon 282 · Oral squamous cell carcinoma · OSCC · Allele specific oligonucleotide · ASO

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Introduction

Oral squamous cell carcinoma (OSCC) is the 12th most common cancer worldwide (8th in males and 13th in females) [1]. Many risk factors can promote oral carcinogenesis, which requires a transition from normal mucosa to precancerous lesions and invasive squamous cell carcinoma [2, 3]. Alteration of the tumor-suppressor gene *P53* is also involved [4]. Many mutations (20–70%) in *P53* are known to cause loss of function in this gene in OSCC [4] and approximately 90% of *P53* mutations are missense mutations [5].

The *P53* gene resides at chromosomal location 17p13.1 and encodes the p53 protein, expression of which can be induced by radiation, chemicals or toxin-induced DNA repair. The p53 protein functions in many cellular biological processes including apoptosis, cell-cycle checkpoint and checking of

DNA integrity before starting cell division [6]. Mutation of *P53* causes loss of wild-type function and accounts for 62.1–92.0% of somatic mutation in various cancers [7]. In addition, mutations within the DNA-binding domain at hot-spot codons R175, G245, R248, R249, R273 and R282 can lead to gain of novel oncogenic functions (GOF) [8].

Human papillomavirus (HPV) infection is an independent risk factor for OSCC. Viral gene products can inactivate many tumor-suppressor proteins, particularly p53, via HPV E6-mediated proteolytic degradation. Many studies have demonstrated that *P53* mutation is rarely or not correlated with HPV-associated cancers including cervical, esophageal and prostate cancers [9–11]. Recently, there have been reports of *P53* mutations in HPV16 E6/E7-immortalized oral epithelial cells at passage 136 compared with HPV-negative normal oral cells [12]. *P53* mutation was also found in association with HPV33 infection in human primary carcinoma of the cervix and cervical intraepithelial neoplasia [13]. These findings may indicate that HPV infection can inactivate p53 protein function not only by degradation, but also by causing *P53* mutation. Many studies have reported a relationship between *P53* mutation and HPV-associated OSCC. However, information about the situation in northeast Thailand is still limited. The population of this region has unique behaviors and risk factors and there has been an increasing prevalence of HPV in OSCC cases from 10% in 2005 to 58% in 2010 [14].

The main exons of *P53* in which mutations occur vary according to geography. For example, exon 6 is the most common exon exhibiting mutations in American OSCC patients (9.3%) [15]. However, mutations in exon 8 predominate among oral submucous fibrosis or OSCC patients in Sri Lankan (28.6%), German (11.7%) and Swedish (36.3%) patients [16–18]. Interestingly, shorter survival was typical for patients bearing a mutation in *P53* exon 8 codon 282. This mutation also significantly upregulated drug-metabolizing enzymes, including the CYP3A4 cytochrome P450, and subsequently drove resistance to several chemotherapeutic drugs [8].

As mentioned above, *P53* exon 8 mutations are associated with HPV infection [12, 13]. However, it is debated whether *P53* mutation pays a role in HPV-associated OSCC and there has been no previous investigation of *P53* codon 282 in association with OSCC. This study is the first in northeast Thailand to investigate *P53* mutation exon 8, especially codon 282, in OSCC and to evaluate relationships between this and HPV status as well as histopathological grade of OSCC.

Materials and Methods

Clinical and Biological Specimens

Exfoliated oral cells from the buccal mucosa of age/sex matched controls (30 individuals) and oral lesion cells (46 samples) of OSCC cases (16 HPV-positive cases and 30 HPV-negative cases) were collected as part of a hospital-based case-control study conducted in northeastern Thailand. Formalin-fixed paraffin-embedded (FFPE) tissues of OSCC patients (60 cases) were also studied. These 60 cases had been diagnosed using histopathology at hospitals in Khon Kaen Province between 2005 and 2010 and comprised of 33 HPV-positive and 27 HPV-negative cases. Mutations in p53, exon 8, codon 282 were investigated among the three sample groups. This study was approved by the Khon Kaen University Ethics Committee in Human Research (no. HE561407 and no. HE 581211).

Preparation of DNA Samples from Exfoliated Cells and Oral Lesion Cells

Each cell pellet was collected and washed by centrifugation at 1,500 rpm for 5 min. Genomic DNA was isolated using Gentra Puregene DNA extraction kit (Qiagen, Hilden, Germany) according the manufacture's guidelines. Cells were lysed by addition of 300 μl of cell lysis solution and the tube was incubated at 65 °C for 1 h. RNA was removed by addition of 1.5 μl RNase A and then incubated at 37 °C for 15 min. Protein was eliminated using protein precipitation solution and then the tube was centrifuged at 13,000 x*g* for 3 min. The DNA pellet was precipitated by isopropanol and the tube subsequently centrifuged at 13,000 x*g* for 5 min, and the pellet washed with 70% ethanol. Dried DNA was dissolved with DNA hydration solution.

Preparation of DNA from FFPE Tissue

Five or six sections (5 μm thickness) cut from each FFPE sample were used for DNA extraction. The sections were deparaffinized and rehydrated by immersion in xylene and 100%, 95% and 70% ethanol, sequentially. QIAamp® DNA Mini Spin Column Kits (Qiagen, Hilden, Germany) were used to extract genomic DNA from FFPE samples as described by the manufacturer. Briefly, cells were lysed by adding 180 μl of Buffer ATL and 20 μl of 20 mg/ml proteinase K and incubated overnight at 56 °C. RNA and protein were removed by addition of 4 μl of 100 mg/ml RNase A and 200 μl Buffer AL, respectively. DNA was pelleted by addition of 200 μl ethanol followed by vortexing. The mixture was carefully transferred into a QIAamp Mini spin column and then centrifuged at 6,000 x*g* for 1 min. DNA was washed with Buffers AW1 and AW2, sequentially. Dried DNA was eluted from the column by addition of 100 μl Buffer AE.

Qualitative Check of the Extracted DNA

Integrity of all DNA samples was checked by NanoDrop 2000 (Thermo Scientific, Rockford, IL, USA) and confirmed by

amplification of a housekeeping human gene, beta actin (β-actin), using specific primers: 5′-TCACCCACACTGTG CCCATCTACGA-3′ and 5′-CAGCGGAACCGCTC ATTGCCAATGG-3′ [19]. The amplification reaction was performed in 25 μl containing 1x PCR buffer, 10 mM of deoxynucleotide triphosphates (dNTP), 2 mM $MgCl_2$, 10 mM of forward and reverse primer, DNA template (20–100 ng) and 0.25 units of Taq DNA polymerase. The PCR reaction used the following program: initial denaturing at 95 °C for 5 min, then 40 cycles consisting of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. Amplification products were separated by 1.5% agarose gel electrophoresis, followed by staining with ethidium bromide, and visualized under an ultraviolet transilluminator.

Detection of *P53* Gene Exon 8 by PCR Analysis

Total DNA from all samples were used to detect *P53* gene exon 8 status by PCR. A total reaction volume of 25 μl PCR master mix containing 1x PCR buffer, 1 mM of dNTPs, 2.5 mM $MgCl_2$, DNA sample (20–50 ng), 0.25 units *Taq* DNA polymerase (Invitrogen) and 10 mM of P53 exon 8 forward and reverse primers; Exon 8 forward; 5′-AGTG GTAATCTACTGGGACGG-3′, Exon 8 reverse; 5′-ACCT CGCTTAGTGCTCCCTG-3′. The PCR reaction started with initial denaturation at 95 °C for 5 min. This was followed by 40 cycles consisting of: denaturation at 95 °C for 1 min, annealing at 58 °C for 1 min, extension at 72 °C for 30 s and a final extension at 72 °C for 4 min. The PCR products (134 bp) were electrophoresed in 2% agarose gels and stained with ethidium bromide. Separated bands were visualized under an ultraviolet transilluminator. SiHa (wild-type *P53*) and C33A (mutant *P53* codon 273) were used as control [20, 21].

The Status of *P53* Gene Exon 8 Codon 282 Assessed by Allele-Specific Oligonucleotide (ASO) Real-Time PCR Analysis

DNA from *P53* exon 8-positive samples was used to determine the status of *P53* (wild type or mutant) at exon 8 codon 282 using ASO-RT-PCR. In this technique, primers are designed with 3′ end matches complementary to either wild-type or mutant sequence. The primer can be extended or not by the DNA polymerase depending on which alternative single base is present in the target sequence. Therefore, only target DNA complementary to the 3′ end of the primer will be amplified.

The ASO-RT-PCR specific for *P53* exon 8 codon 282 was performed in a total reaction volume of 20 μl containing 10 μl of EvaGreen (SsoFast™ EvaGreen® Supermix, Biorad Laboratories Inc., Hercules, CA, USA), 0.3 μl forward primer, 0.3 μl reverse primer, 7.4 μl of distilled water and 50–100 ng of DNA template. Primers used were as follows; P53WT forward primer; 5′-CTTGTCCTGGGAGAGACC-3′ for P53 wild type, P53MU forward primer; 5′-CTTG TCCTGGGAGAGACT-3′ for the R282W P53 mutation in exon 8 (C > T) and P53 reverse (common) primer; 5′-GAGC TGGTGTTGTTGGGC-3′ for both P53 wild type and mutants [22]. Amplification was performed in duplicate (differing in which forward primer was used) at a final volume of 20 μl/well in a 96-well PCR plate using the LightCycler 480 real-time PCR instrument (Roche Diagnostics, Indianapolis, IN, USA). The reaction was subjected to enzyme activation at 98 °C for 2 min, followed by 40 cycles of denaturation at 98 °C for 5 s, annealing at 58 °C (wild type) or 55 °C (mutant) for 20 s, extension at 72 °C for 20 s and melting curve analysis at 65–95 °C for 5 s/step. Total DNA derived from SiHa cells was used as control for *P53* wild type and DNA derived from C33A was used similarly for *P53* mutation. A negative control was included in each experiment to ensure reproducible results. The RT-PCR products were visualized under a UV-transilluminator with ethidium bromide on a 2% agarose gel.

Statistical Analysis

The proportion of mutations in *P53* exon 8 codon 282 in OSCC oral lesion cells and FFPE tissues was compared to controls using binary logistic and multinomial logistic regression using SPSS 13.0 for Windows (SPSS Inc., Chicago, USA) and Prism 5 for Windows (GraphPad Software Inc., San Diego, CA, USA).

Results

Detection of *P53* Gene Mutation at Exon 8

The *P53* gene status at exon 8 was evaluated in exfoliated cells from controls (30 samples) and OSCC patients which consisted of oral lesion cells (46 samples) and FFPE OSCC tissues (60 samples). Frequency of mutation at exon 8 was 69.6% (32/46) in OSCC oral lesion cells, significantly higher than in exfoliated cells from controls (16.7%, 5/30) as shown in Table 1. These 5 control samples with *P53* gene mutation at exon 8 had risk factors including exposure with smoking, betel quid chewing and/or alcohol consumption as previously described [23]. In addition, a high frequency (81.7%, 49/60) of mutations was also found in FFPE oral tissues. Correspondingly, wild-type p53 was infrequent in OSCC samples but frequent in exfoliated cells from controls. These results demonstrated that mutation of *P53* exon 8 was common in OSCC cases in northeast Thailand.

Table 1 Mutation of *P53* exon 8 in OSCC samples and in controls

	P53 exon 8 mutation	Wild type	*P* value	Odds	95% CI
Controls					
Exfoliated cells ($n = 30$ samples)	5 (16.7%)	25 (83.3%)	Ref.	Ref.	Ref.
OSCC					
Oral lesion cells ($n = 46$ samples)	32 (69.6%)	14 (30.4%)	< 0.001	11.429	3.628–35.998
FFPE tissue ($n = 60$)	49 (81.7%)	11 (18.3%)	< 0.001	22.273	6.970–71.170

OSCC samples were divided into two groups, oral lesion cells and FFPE tissue samples

Detection of *P53* Exon 8 Mutation in HPV-Positive and -Negative OSCC Samples

P53 exon 8 mutation was investigated in HPV-positive and -negative OSCC samples. Mutations were present in 62.5% (10/16) and 81.8% (27/33) of HPV-positive oral lesion cells and FFPE tissues, respectively. The proportion of samples exhibiting mutation did not differ significantly between HPV-positive and -negative cases (Table 2). This applied both to oral lesion cells and FFPE tissues. This result demonstrated that the proportion of mutation of *P53* exon 8 in HPV-positive was not different from negative OSCC.

P53 Gene Status in Exon 8 Codon 282 in OSCC

Frequent missense mutations of the *P53* gene in human cancer have been reported. These occur in DNA-binding domains at residues R175, G245, R248, R249, R273 and R282. In this study, aberrant codon 282 in exon 8 was investigated by ASO RT-PCR using EvaGreen fluorescent dye. Real-time PCR amplification signals were obtained, and melting peaks were detected for wild type (Tm = 84 °C) and mutant type (Tm = 81 °C) (Fig. 1a and b). The ASO RT-PCR products were run on a 2% agarose gel and visualized under a UV light with ethidium bromide. The products had a band size of 207 base pair as shown in Fig. 1c.

Mutation of *P53* exon 8 codon 282 was frequently found in OSCC patients including 32.6% (15 of 46 cases) in oral lesion cells and 35% (21 of 60 cases) in FFPE tissue samples but was absent in controls. This mutation was significantly associated with OSCC (odds = 1.484, 95% CI = 1.214–1.814 and $P < 0.001$ in oral lesion cells and odds = 1.538, 95% CI = 1.278–1.8 and $P < 0.001$ in FFPE OSCC tissues) as shown in Table 3. This result demonstrated that mutation of *P53* at codon 282 might play an important role of oral carcinogenesis in northeast Thailand.

Mutation of *P53* Exon 8 Codon 282 in HPV-Positive and -Negative OSCC

A relationship between mutation of *P53* exon 8 codon 282 and HPV in OSCC has never been reported. Table 4 shows no significant relationship between HPV infection and mutation, either in oral lesion cells or FFPE tissues. This information may indicate that HPV infection may not strongly affect frequency of *P53* codon 282 mutation in OSCC.

Mutation of *P53* Exon 8 Codon 282 in OSCC Tumors of Different Histopathological Grades

It is well known that patients with well-differentiated OSCC have the best prognosis, relative to those with moderately and poorly differentiated tumors [24, 25]. HPV infection is

Table 2 Mutation frequencies of *P53* exon 8 in HPV-positive and -negative OSCC samples

Sample	*P53* exon 8 mutation, n (%)		*P* value	Odds	95%CI
	Positive	Negative			
Oral lesion cells, $n = 46$					
HPV-positive, $n = 16$	10 (62.5)	6 (37.5)	0.447	0.606	0.166–2.215
HPV-negative, $n = 30$	22 (73.3)	8 (26.7)			
FFPE tissues, $n = 60$					
HPV-positive, $n = 33$	27 (81.8)	6 (18.2)	0.973	1.023	0.275–3.804
HPV-negative, $n = 27$	22 (81.5)	5 (18.5)			

95% CI indicates 95% confident intervals

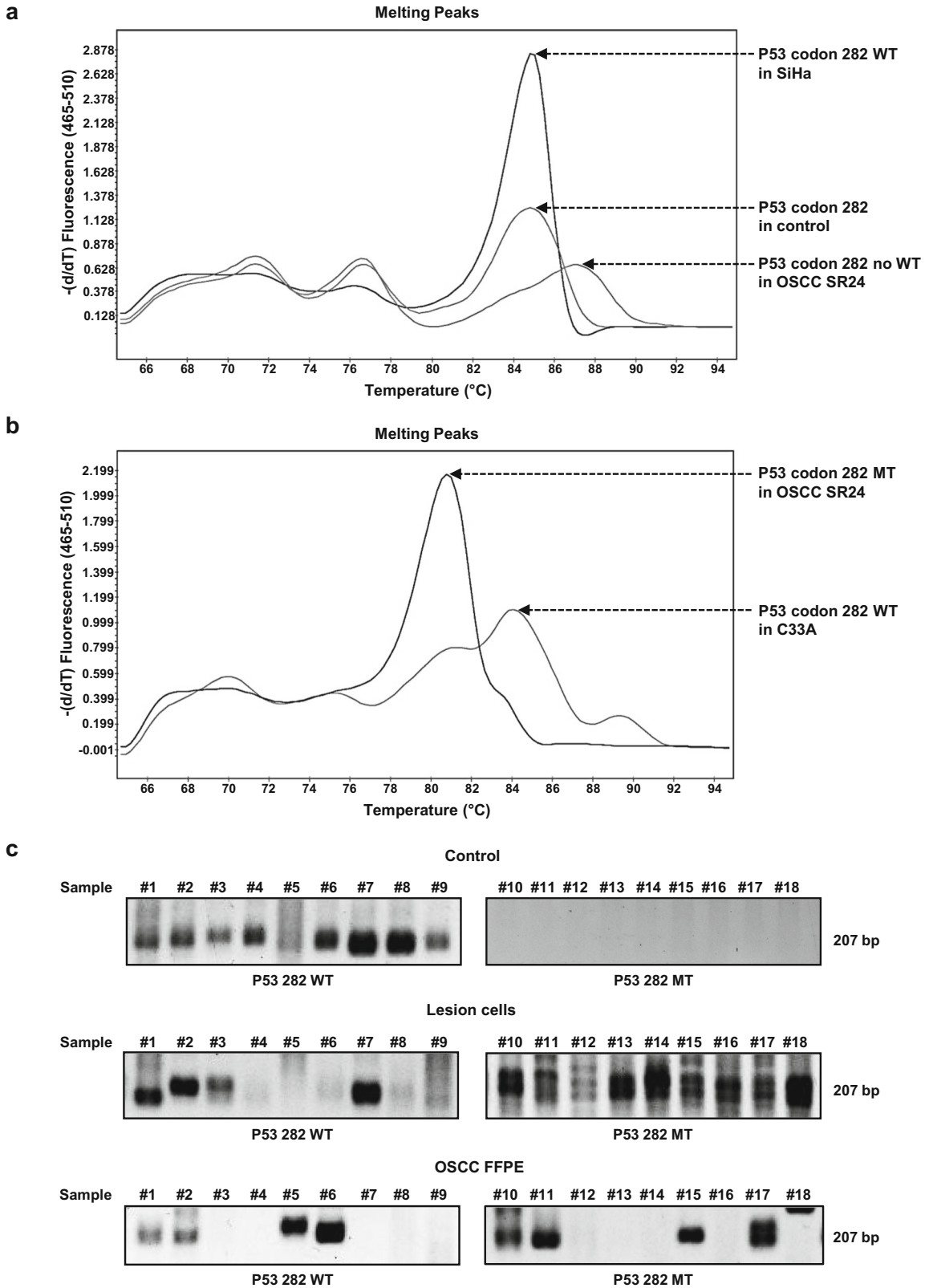

Fig. 1 Representative traces showing ASO RT-PCR melting peaks for PCR products including *P53* exon 8 codon 282 derived from control, oral lesion cells and FFPE OSCC samples. The melting peak of wild type (Tm = 84 °C) (**a**) is shown in the left panel and that of the mutant (Tm = 81 °C) (**b**) is shown in the right panel. Gel electrophoresis of ASO RT-PCR products of exon 8 codon 282 (207 bp) was separated by 2% agarose electrophoresis among control, lesion cells and FFPE OSCC samples. The left and right panels represent the PCR product from *P53* codon 282 wild type (WT) and mutant (MT), respectively

Table 3 *P53* exon 8 codon 282 mutation status

Sample	*P53* mutation present in exon 8 codon 282	*P* value	Odds	95% CI
Control				
Exfoliated cells from controls ($n = 30$)	0 (0%)	Ref.	Ref.	Ref.
OSCC				
Oral lesion cells ($n = 46$)	15 (32.6%)	< 0.001	1.484	1.214–1.814
FFPE tissues ($n = 60$)	21 (35.0%)	< 0.001	1.538	1.278–1.852

Table 4 Prevalence of *P53* exon 8 codon 282 mutation in HPV-positive and -negative OSCC cases

Sample	*P53* mutation in exon 8 *P53* exon 8 codon 282	*P* value	Odds	95% CI
Oral lesion cells ($n = 46$)	15 (32.6%)			
HPV-negative ($n = 30$)	11 (36.7)	–	–	–
HPV-positive ($n = 16$)	4 (25)	0.424	0.576	0.149–2.229
FFPE tissues ($n = 60$)	21 (35.0%)			
HPV-negative ($n = 27$)	8 (29.6)	–	–	–
HPV-positive ($n = 33$)	13 (39.4)	0.431	1.544	0.523–4.553

associated with well-differentiated OSCC [26]. Mutation of *P53* exon 8 codon 282 is significantly associated with poor prognosis in various cancers, but this has not been reported in OSCC [8]. In this study, frequency of this mutation was not significantly different between well differentiated (38.9%) and moderately/poorly differentiated OSCC (30.4%) as shown in Supplementary Table 1.

Mutation of *P53* Codon 282 in Relation to Histopathological Grades and HPV Infection Status

In our study, HPV was more commonly detected in well-differentiated (58.3%) than moderately/poorly differentiated OSCC tissues (52.2%) (Supplementary Table 2). The mutation in *P53* codon 282 was correlated with tumor histopathological classification and HPV status as shown in Fig. 2. Nine out of 32 (28.1%) HPV-positive cases exhibited the *P53* mutation in well-differentiated OSCC, whereas only 4/27 (14.8%) HPV-negative cases carried this mutation. In moderately/poorly differentiated HPV-positive OSCC, 4/32 (12.5%) cases exhibited the mutation: for HPV-negative cases, the same percentage and number (14.8%, 4/27) carried the mutation. The *P53* codon 282 mutation was most frequent in well-differentiated HPV-positive OSCC.

Discussion

This is the first study of frequencies of mutation of *P53* exon 8 codon 282 in OSCC patients in relation to HPV infection and

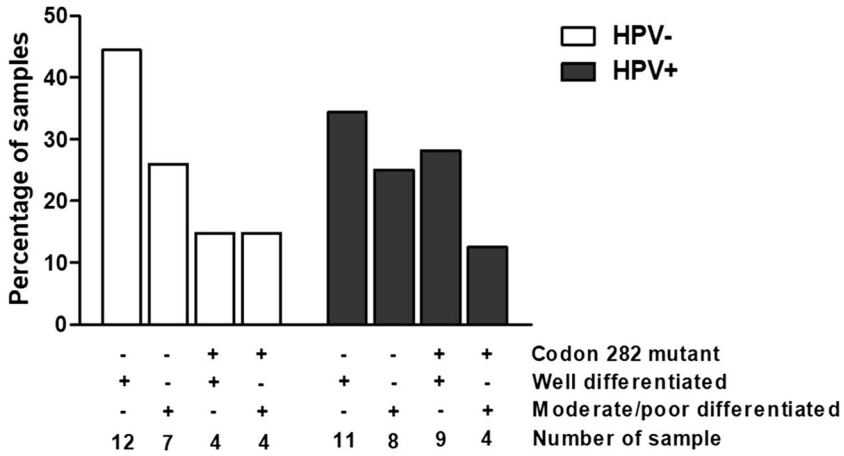

Fig. 2 Percentage of HPV-positive and -negative OSCC samples in relation to *P53* codon 282 mutation and histopathological grade. The concurrent detection of *P53* mutation codon 282 status with histopathological classification was investigated between HPV-positive ($n = 32$) and negative ($n = 27$) samples

tumor histopathological grade. We found a high prevalence of this mutation (69.6–81.7%) among northeastern Thai OSCC patients. Mutations at this hot-spot range from frequencies of 11.7% to 66.7% among OSCC patients worldwide [16–18, 27]. Mutational frequencies in other exons vary. For instance, mutations were most common in *P53* exons 5 (40%) and 6 (40%) in non-smoking American OSCC patients [28]. Mutations in exon 5 were detected in 47.6% of Southern Thai OSCC patients with a history of smoking and alcohol drinking followed by betel quid chewing combined with smokeless tobacco (30%) and betel quid alone (20%) [29]. Mutations in *P53* exon 8 were found in 66.7% of Taiwanese OSCC patients. All of the patients exhibiting mutations were smokers, and 50% used betel quid [27].

The mutant of the *P53* gene that we studied (R282W) results in an arginine being replaced by a tryptophan at codon 282. This codon is located at the binding surface to Bcl-XL. It is highly associated with shorter survival periods and confers GOF effects including inhibition of pro-apoptotic transcription and regulation of non-coding RNA, and is a promising biomarker for cancer prognosis [30]. This hot spot was the most frequent site of mutation in Indian non-small cell lung cancer [22]. In OSCC, the reported prevalence of codon 282 mutation is low, 1.1%, 2.2% and 4% in German, Taiwanese and Swedish patients, respectively [17, 18, 31]. By contrast, we found this mutant in 32.6% and 35.0% of oral lesion cells and FFPE tissues from OSCC samples, respectively. Moreover, we found the mutation of *P53* exon 8 in 5 samples from exfoliated cells in controls and all of these had history of exposure to smoking, alcohol drinking and/or betel quid chewing. These risk factors are associated with increased the incidence of *P53* mutation in OSCC [32–34]. Interestingly, deletions between codons 287 and 292 of exon 8 of the *P53* gene were significantly associated with female Swedish OSCC patients [17]. However, we found no gender bias among Thai patients (data not shown). These various results may be due to differences in techniques used, unique behaviors of different ethnic groups, geography, number of samples, type of samples, etc.

HPV has been found in over 90% of cervical cancer samples, but *P53* mutation is apparently very rare [35]. An inverse association between the presence of *P53* mutations and HPV16 infection in tissue specimens from OSCC cases has been found in various countries (Italy, Spain, Northern Ireland, Poland, India, Cuba, Canada, Australia and the Sudan); unsurprisingly, *P53* mutation was found more frequently in OSCC samples from smokers than from non-smokers [33, 36]. On the other hand, expression of HPV16 and 18 E6/E7 (i.e. HPV infection) was significantly associated with *P53* gene mutation in Southern Japanese OSCC cases: 30.4% had HPV infection and this mutation [37]. We found that the frequency of the *P53* exon 8 mutation (62.5% and 81.8%) in HPV-positive OSCC oral lesion cells and FFPE tissues did not differ from HPV-negative samples (73.3 and 81.5%), respectively. This might suggest that mutation of *P53* exon 8 is not directly associated with HPV infection in this region. In this study, we also report for the first time that the proportion of codon 282 mutations (25% and 39.4%) in HPV-positive samples is similar to that in HPV-negative samples (36.7% and 29.6%) (Table 4). This mutation might be induced by the processes of carcinogenesis that may be involved in the exposure to risk factors including smoking, alcohol consumption and particularly betel quid chewing, which is the main risk factor in our region. Oda et al., demonstrated that recombinant retrovirus containing HPV16 E6/E7-transduced normal human oral epithelial cells were immortalized and consequently found *P53* exon 8 codon 273 mutation at passage 136, but not in normal oral epithelial cells, which suggested that HPV16 may be capable of inactivating the *P53* gene by causing it to mutate [12].

The *P53* mutation was significantly associated with poorly differentiated OSCC in USA and India [28, 38]. Conversely, HPV-associated OSCC was correlated with the well-differentiated grade in many regions including northeastern Thailand [19]. Consistent with our findings, *P53* exon 8 mutation in Southern Thai OSCC patients was mostly found in well-differentiated OSCC [29]. There have been many reports of a significant association between HPV-infected OSCC and a good prognosis in USA, Japan, Sweden and Czech Republic [39–42]. Conversely, HPV-infected OSCC in Israel was significantly associated with shorter survival time when compared with HPV-negative cases [43]. Interestingly, presence of HPV in head and neck cancer together with *P53* mutation was significantly associated with the shortest survival times in the USA [44]. Given these disparate results, the association between *P53* mutation and prognosis in OSCC remains unclear. In our study, the co-incidence of *P53* exon 8 codon 282 mutation and HPV infection was higher in well-differentiated OSCC (28.1%) than in HPV-negative cases (14.8%) (Fig. 2). This finding might be useful for prognosis prediction.

As mentioned above, mutations in *P53* exon 8 were more strongly associated with poor outcomes and metastasis than were those in other exons in Japanese [45] and Taiwanese non-small cell lung cancer [46]. Therefore, the co-incidence of this mutation and HPV infection may increase the risk of poor outcomes [44]. Although HPV-negative OSCC cases have a poorer prognosis than HPV-positive cases [40], many researchers have demonstrated poorer outcomes in HPV-infected patients bearing *P53* mutations than in patients with HPV infection only [47]. However, HPV-negative cases may have a significantly poorer prognosis than HPV-positive cases with or without *P53* mutation [40]. Therefore, information concerning the status of *P53* exon 8 codon 282 and HPV infection in OSCC may be useful for evaluating prognosis in northeastern Thailand. The main limitation of our study was the small sample size employed. Our results may therefore not

be an accurate picture of the actual epidemiology of this hot spot of mutation and HPV infection in our region. However, this information may open a new perspective for informing therapy in northeast Thai OSCC patients in the future.

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Compliance with Ethical Standards

Ethics Approval and Consent to Participate All procedures performed in studies involving human participants were approved by the Khon Kaen University Ethics Committee for Human Research (No. HE561407 and No. HE581211), in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study formal consent is not required.

Conflict of Interest The authors have no conflict of interest.

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