Expression of prion protein is closely associated with pathological and clinical progression and abnormalities of p53 in head and neck squamous cell carcinomas

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Received August 2, 2015; Accepted October 20, 2015

DOI: 10.3892/or.2015.4425

Abstract. Prion protein (PrP) is a glycosyl-phosphatidylinositol (GPI)-anchored membrane protein that functions as a unique pathogenic agent in transmissible spongiform encephalopathy (TSE). In the past decade, overexpression of PrP was observed in a number of human malignant tumors, such as gastric, breast and pancreatic cancer. However, the role of PrP expression in squamous cell carcinoma is rarely documented. To screen PrP expression in head and neck squamous cell carcinoma (HNSCCs), the paraffin-embedded specimens of 92 pathologically diagnosed HNSCCs were assessed by PrP-specific immunohistochemistry (IHC). A total of 55.43% (51/92) of the tested carcinoma tissues were PrP-positive. The rate of positivity and the staining intensity of PrP were closely related with the pathological degree of the HNSCCs; a higher rate of PrP expression was noted in the group of poorly differentiated cancers. PrP-positivity rates increased along with the progression of the clinical grade of the carcinomas. Further evaluation of the associations between PrP expression and the data concerning p53 abnormalities and human papillomavirus (HPV) infection in these samples as previously described, revealed that PrP-positive staining was more frequently detected in the tissues with p53-positive accumulation and the wild-type *TP53* gene. The patients with a proline (Pro) polymorphism in SNP72 of TP53 showed significantly higher PrP-positive rates than those with arginine (Arg). No notable difference in PrP expression was identified between the HPV-positive and HPV-negative group. These data indicate a close association of PrP expression with clinical and histological differentiation of HNSCCs, as well as abnormalities of p53.

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Key words: PrP, HNSCC, p53, HPV

Introduction

Head and neck squamous cell carcinoma (HNSCC) is a group of common malignant cancers located in the oral cavity, hypopharynx, oropharynx and larynx (1). Annually, HNSCCs account for more than 550,000 cases of cancer worldwide (2). Research suggests that tobacco use and alcohol consumption are two major risk factors for HNSCC. Human papillomaviruse (HPV) infection, particularly high-risk HPVs, are closely associated with HNSCCs, particularly carcinomas of the lip and oral cavity (3,4). The tumor-suppressor p53, which plays essential roles in many different types of human malignant tumors, was also found to be associated with the occurrence of HNSCC (5). Various types of mutations in the p53-encoding gene (*TP53*) have been identified in HNSCC tissues (6). In addition, various single-nucleotide polymorphisms (SNPs) in *TP53*, such as the polymorphism in codon 72 (SNP72),

significantly influence the age at onset of various types of HNSCCs (7).

Prion protein (PrP) is a copper binding glycoprotein, which is highly conserved throughout evolution (8). PrP exists in many types of tissues, but is mostly concentrated in the central nervous system (CNS). PrP is directly involved in prion diseases or transmissible spongiform encephalopathies (TSEs) that are a group of fatal neurodegenerative diseases, such as human Creutzfeldt-Jakob disease (CJD), scrapie and bovine spongiform encephalopathy. In these cases, normal cellular PrP (PrP^C) is converted into a proteinase-resistant and pathological isoform (PrP^{Sc}) (9). As a cellular membrane protein, normal PrP is expressed in the neurons and glia of the brain and spinal cord, as well as in several peripheral tissues and in leukocytes (10). The efficient transcription of PrP is detectable in the brains of mice and chickens beginning early in embryogenesis, and its level increases as development proceeds (11). Although its wide expression in many cell types indicate a more cosmopolitan biological function, the exact cellular function remains undefined. Evidence suggests that PrP^C is important for synaptic activity, cell adhesion and recognition, ligand uptake, transmembrane signaling and neuroprotection (12). The biological function of PrP in peripheral tissues has been rarely addressed.

In the past decade, the association of PrP with human malignant tumors has attracted great attention. PrP has been found to be overexpressed in a variety of cancers including gastric, pancreatic and breast cancers, osteosarcoma and melanoma (13-15). Various studies have even proposed that overexpression of PrP is closely associated with the poor prognosis of pancreatic and breast cancers, highlighting that it may affect the growth and invasiveness of cancers (16). PrP expression is also believed to play an important role in the acquisition of multidrug-resistant (MDR) gastric cancer (17). However, the expression of PrP and its role in HNSCCs remain undescribed.

In the present study, PrP expression in tumor tissues from 92 pathologically diagnosed HNSCC cases were screened with PrP-specific immunohistochemical assays. We found that 55.43% (51/92) of the tested carcinomas were PrP-positive. The positive rate and the staining intensity of PrP were closely related with the pathological degree of the tumors. Additionally, PrP-positive rates also showed correlations with the anatomic site and the clinical grade of the carcinomas. Further evaluation of the associations of PrP expression with p53 abnormalities and HPV infection in malignant tissues revealed that PrP-positive staining was more frequently detected in the tissues with p53-positive accumulation and the wild-type *TP53* gene.

Materials and methods

Specimens. The paraffin-embedded specimens of HNSCCs used in our previous studies (7,18) were employed in the present study, except one laryngocarcinoma case without further available tissue. Totally, 92 HNSCCs were recruited, including 63 laryngocarcinoma, 5 oropharyngeal, 15 hypopharynx and 9 lip carcinomas. All patients were hospitalized at the Department of Head and Neck Surgery, Peking University Cancer Hospital and Institute, from 2006 to 2011. The demographic, clinical and pathological information of these patients was previously described (18). Six surgically removed tissues from the patients with laryngocarcinoma were stored at -80˚C.

Immunohistochemistry (IHC). Paraffin sections (5-μm in thickness) were deparaffinized in xylene for 5 min twice and gradually routinely rehydrated. The sections were quenched for endogenous peroxidases in 3% H_2O_2 in methanol for 15 min, and pretreated with enzyme digestion antigen retrieval for 1 min. After blocking in 1% normal goat serum, the sections were incubated overnight at 4˚C with 1:500-diluted PrP-specific mAbs, including 3F4 (MAB1562; Millipore), 6D11 (sc-58581), 7D9 (sc-58582) (both from Santa Cruz Biotechnology) and 8H4 (ab-61409; Abcam). The sections were then incubated for 60 min with 1:1,000-diluted HRP-conjugated goat anti-mouse secondary antibody (Vector Labs, USA), and visualized by incubation with 3,3-diaminobenzidine tetrahydrochloride (DAB). The slices were dehydrated and mounted in Permount. Photomicrographs were captured with a DP70 digital camera mounted on a BX5 microscope (Olympus Optical, Japan).

Determination of the degree of PrP-positive staining. The strategy for determination of the degree of PrP-positive staining in the tested tissues was based on a protocol described elsewhere (19). Briefly, five fields under microscopy were randomly selected for each slice. The score was given based on the ratio of positively stained cells and gradation of the stained color. A total of <10% positive cells was recorded as 0, 10-25% as 1, >25-50% as 2, >50-75% as 3, and >75% as 4. No positive staining was scored as 0, light brown signals in cells was scored as 1, brown signals in cells was scored as 2, deep brown signals was scored as 3. The score of the percentage of positive cells and the score of the gradation of the stained color was multiplied and the final result was given based on the product: 0-1 as negative (-), 2-4 as weakly positive (+), 5-8 as positive (++), 9-12 as strongly positive (+++).

Preparation of tissue homogenates. The tissue samples of HNSCC and the brain samples of normal and scrapie-infected mice were homogenized in 10% lysis buffer (w/v, 100 mM NaCl, 10 mM EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 10 mM Tris, pH 7.5) according to a protocol described elsewhere (20). Then, tissue debris was removed with low speed centrifugation at 2,000 x g for 10 min and the supernatants were collected for further study.

Ethics statement. Written consent for further investigation and publication was obtained from the patients or the patients' relatives, respectively. Usage of the stored human samples in the present study was approved by the Ethics Committees of Peking University Cancer Hospital and the Institute and National Institute for Viral Disease Prevention and Control, China CDC.

Statistical analysis. Statistical analysis was performed using Chi-square and Fisher's exact tests for correlations between groups for HPV infection, SNP72 type, p53 mutation and IHC. Mann-Whitney method was used for the relationship between SNP72 and age. A probability value of <0.05 was considered to indicate a statistically significant result. All statistical analyses were performed by SPSS 20 (IBM, USA).

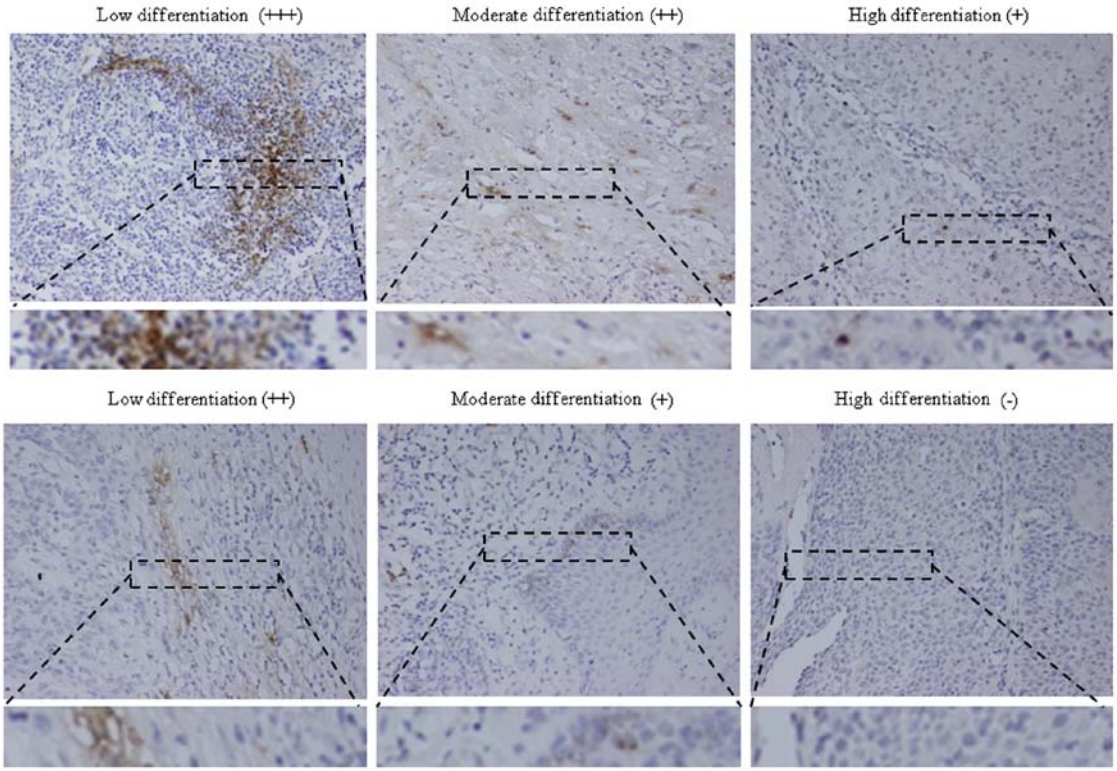

Figure 1. Immunohistochemical assays of PrP proteins in the tissues of head and neck squamous cell carcinomas of various pathological grades (magnification, x40). The magnified views are shown below each image (magnification, x100). The pathological grade is indicated at the top of each image.

Table I. PrP-positive staining in the HNSCC cases.

Site	No. of cases	PrP				Positive rate (%)
		- n (%)	+ n (%)	++ n (%)	+++ n (%)	
Lip	9	5 (55.56)	2 (22.22)	2 (22.22)	0 (0.00)	44.44
Oropharynx	5	2 (40.00)	2 (40.00)	0 (0.000)	1 (20.00)	60.00[a]
Hypopharynx	15	8 (53.33)	5 (33.33)	2 (13.33)	0 (0.00)	46.67[b]
Larynx	63	26 (41.27)	32 (50.79)	5 (7.94)	0 (0.00)	58.73[c]
Total	92	41 (44.57)	41 (44.57)	9 (9.78)	1 (1.09)	55.43

[a]P=0.0015 compared with lip carcinoma. [b]P=0.6549 compared with lip carcinoma; P=0.0065 compared with hypopharynx carcinoma. [c]P=0.0037 compared with lip SCC. P=0.796436 compared with oropharyngeal SCC; P=0.0143 compared with hypopharynx SCC. PrP, prion protein; HNSCC, head and neck squamous cell cancer.

Results

PrP expression is detectable in more than half of the carcinoma tissues. PrP protein is widely expressed in CNS. To ascertain the expression of PrP in HNSCC tissues, several commercial PrP monoclonal antibodies were selected and screened for the potential usage in the IHC assays: mAbs 3F4, 6D11, 7D9 and 8H4. Using the sections from the same paraffin-embedded tissues of 3 different patients, the immunoreactivities of the mAbs were comparably evaluated under the same experimental conditions. mAb 3F4 produced the most significant reactivity in the IHC assays, with clear brown positive staining in the cytoplasm and membrane of the carcinoma cells. Therefore, PrP-specific mAb 3F4 was used in the IHC assays in the subsequent tests.

Totally, 92 slices were stained with mAb 3F4 immunofluorescently, and 51 cases (55.43%) were PrP-positive. Based on the judgment protocol described above, 41 (44.57%) cases were weakly positive for PrP (+), 9 (9.78%) were PrP positive (++) and only one case was strongly positive for PrP (+++). As shown in Fig. 1, the PrP-specific staining was concentrated in the carcinoma cells, mostly distributed in the cytoplasm. In some areas, the strongly positive staining formed large positive masses. PrP-positive staining was rarely observed in the regions of morphologically normal tissues.

Table II. PrP-positive staining in HNSCC based on the clinical degrees.

		PrP				
Clinical degree	No. of cases	- n (%)	+ n (%)	++ n (%)	+++ n (%)	Positive rate (%)
I	27	15 (55.56)	9 (33.33)	3 (11.11)	0 (0.000)	44.44
II	35	13 (37.14)	19 (54.29)	3 (8.57)	0 (0.000)	62.86[a]
III	25	11 (44.00)	11 (44.00)	3 (12.00)	0 (0.000)	56.00[b]
IV	5	2 (40.00)	2 (40.00)	0 (0.000)	1 (20.00)	60.00[c]
Total	92	41 (44.57)	41 (44.57)	9 (9.78)	1 (1.09)	55.43

[a]P=0.0001 compared to clinical degree I; [b]P=0.0199 compared to I; P=0.1669 compared to II; [c]P=0.0014 compared to I; P=0.5594 compared to II; P=0.414216 compared to III. PrP, prion protein; HNSCC, head and neck squamous cell carcinoma.

Table III. PrP-positive staining in HNSCC based on the pathological degrees.

		PrP				
Pathological degree	No. of cases	- n (%)	+ n (%)	++ n (%)	+++ n (%)	Positive rate (%)
Highly differentiated	26	18 (69.23)	7 (26.92)	1 (3.85)	0 (0.00)	30.77
Moderately differentiated	51	20 (39.22)	26 (50.98)	5 (9.80)	0 (0.00)	60.78[a]
Lowly differentiated	15	3 (20.00)	8 (53.33)	3 (20.00)	1 (6.67)	80.00[b]
Total	92	41 (44.57)	41 (44.57)	9 (9.78)	1 (1.09)	55.43

[a]P<0.00001 compared to the highly differentiated cases. [b]P<0.00001 compared to the highly differentiated cases. P<0.00001 compared to the moderately differentiated cases. PrP, prion protein; HNSCC, head and neck squamous cell carcinoma.

Among the 92 tested HNSCC specimens, 63 were located in the larynx, 5 in the oropharynx, 15 in the hypopharynx and 9 in the lip. PrP expression was positive in 44.44% (4/9) of the lip carcinomas, 60% (3/5) of the oropharyngeal carcinomas, 46.67% (7/15) of the hypopharynx carcinomas and 58.73% (37/63) of the laryngeal carcinomas (Table I). Statistical analysis showed significantly higher PrP expression in the cancers of the oropharynx and larynx.

Correlation between PrP expression and the clinical degrees of the HNSCCs. To test the potential correlation between PrP-positive staining and the clinical grade at the time of surgical operation, 92 of the HNSCCs were grouped according to their clinical degrees. The PrP-positive rates of clinical degree I, II, III and IV cases were 44.44 (12/27), 62.86 (22/35), 56 (14/25) and 60% (3/5), respectively (Table II). Statistical assays revealed significantly low PrP-positive rates in the group of clinical degree I than that in II (P=0.0001), III (P=0.0199) and IV (P=0.0015). There was no statistical difference in the PrP-positive rate among the tumors of clinical degree II, III and IV. The distributions of the PrP-positive carcinomas based on the intensities were quite comparable. Tumor tissues in later clinical degree had higher PrP-positive rates.

Correlation between PrP expression and the pathological grade of the HNSCCs. To ascertain the possible relationship between PrP expression and pathological differentiation, the tested HNSCCs were divided into groups of low, moderately and highly differentiated carcinomas. The PrP-positive rates were 30.77% (8/26) in the highly differentiated, 60.78% (31/51) in the moderately differentiated and 80% (12/15) in the lowly differentiated HNSCCs, respectively (Table III). Statistical analysis obviously showed significance among the three groups and between each group (P<0.0001). Further analysis identified that 8 out of 9 PrP-positively stained (++) cases were distributed in the groups of moderately and lowly differentiated carcinomas, and one strongly PrP-positive (+++) case was poorly differentiated carcinoma. This strongly indicates a close association of PrP expression with poorly differentiated HNSCCs.

Correlation between the PrP expression and abnormalities of p53 in the HNSCCs. Our previous study proposed that among all 92 HNSCC cases, 37 cases (40.22%) were p53-positive and 55 cases (59.78%) were p53-negative. Sequencing analysis of the *TP53* gene revealed that 34 (36.96%) cases contained various mutations (7). Analysis of the possible relationship between PrP and p53 staining in IHC identified that the rate of PrP-positive staining was 64.86% (24/37) in the p53-positive patients, while the rate was 49.09% (27/55) in the p53-negative patients, showing statistical difference (P=0.0041) (Fig. 2A). All 6 HNSCC cases showing p53 strong positive p53 staining was also PrP-positive. In contrary, positive staining for PrP was detected in 44.11% (15/34) of the patients containing mutations in *TP53* and 62.07% (36/58) of those without a mutation in *TP53*, showing high significance (P=0.0002) (Fig. 2B). This

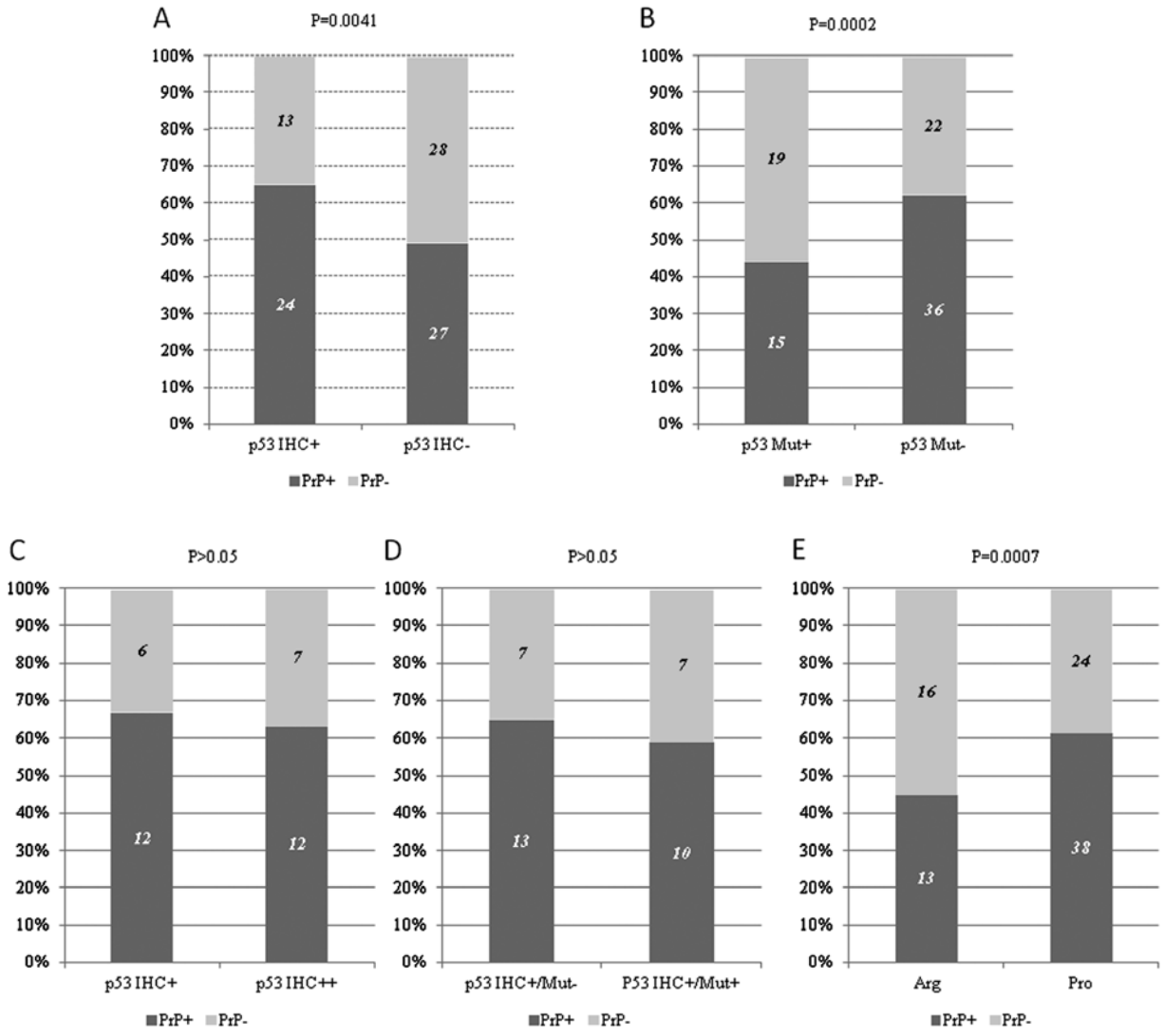

Figure 2. Correlation between PrP expression and abnormalities of p53 in the HNSCCs. (A) The percentage of PrP-positive staining in the patients with p53-positivity and -negativity. (B) The percentage of PrP-positive staining in the patients with *TP53* mutation and without *TP53* mutation. (C) The percentage of PrP-positive staining in the patients with weakly positive p53 (p53 IHC+) and with strongly positive p53 (p53 IHC++). (D) The percentage of PrP-positive staining in the patients whose p53-positivity in IHC with *TP53* gene mutation and without *TP53* gene mutation. (E) The percentage of PrP-positive staining in the patients with Arg/Arg homozygote and with Pro/Pro homozygote or Pro/Arg heterozygote. PrP, prion protein; HNSCC, head and neck squamous cell carcinoma.

indicates that the patients with p53-positive cancer cells and the patients with the wild-type *TP53* gene had a higher possibility to be PrP-positive.

To ascertain the possible linkage of PrP-positive staining with the intensities of the p53-positive staining in the IHC assays, 37 p53-positive cases in IHC were grouped as weakly positive (p53 IHC+) (18 cases) and strongly positive (p53 IHC++) (19 cases). The positive rates of PrP expression in the groups of p53 IHC+ and p53 IHC++ were 66.67% (12/18) and 63.15% (12/19), respectively, without statistical difference in the PrP-positive rate (P>0.05) (Fig. 2C). Furthermore, the cases with PrP-positive expression in the 37 cases with p53-positivity in IHC were analyzed according to whether they had mutations in their *TP53* gene. Out of 17 patients with mutations in *TP53*, 58.82% (10/17) were PrP-positive, while out of 20 patients with wild-type *TP53*, 65.00% (13/20) were PrP-positive (Fig. 2D). Although the PrP-positive rate in the patients with p53-positive staining and mutated *TP53* was slightly lower than in the patients with p53-positive staining and wild-type *TP53*, statistical analysis did not achieve significance (P>0.05). This highlights that either the intensity of p53-positive staining or the status of *TP53* did not notably influence the PrP expression in the group of p53-positive HNSCCs.

Single-nucleotide polymorphisms (SNPs) in codon 72 (SNP72) of *TP53* include arginine (Arg)/Arg, Pro/Pro and Pro/Arg. Among the 91 HNSCCs with data for p53 SNP72, 29 cases were Arg/Arg homozygote whose phenotype is nominated as Arg, 62 were Pro/Pro homozygote or Pro/Arg heterozygote whose phenotype is termed as Pro (7). The distributions of PrP-positive rates in these two groups were obviously different; the PrP-positive rate in the group of Pro/Pro homozygotes or Pro/Arg heterozygotes (61.29%, 38/62) was significantly higher than those of the Arg/Arg homozygote (44.83%, 13/29), indicating a close correlation between PrP expression and p53 SNP72 (Fig. 2E).

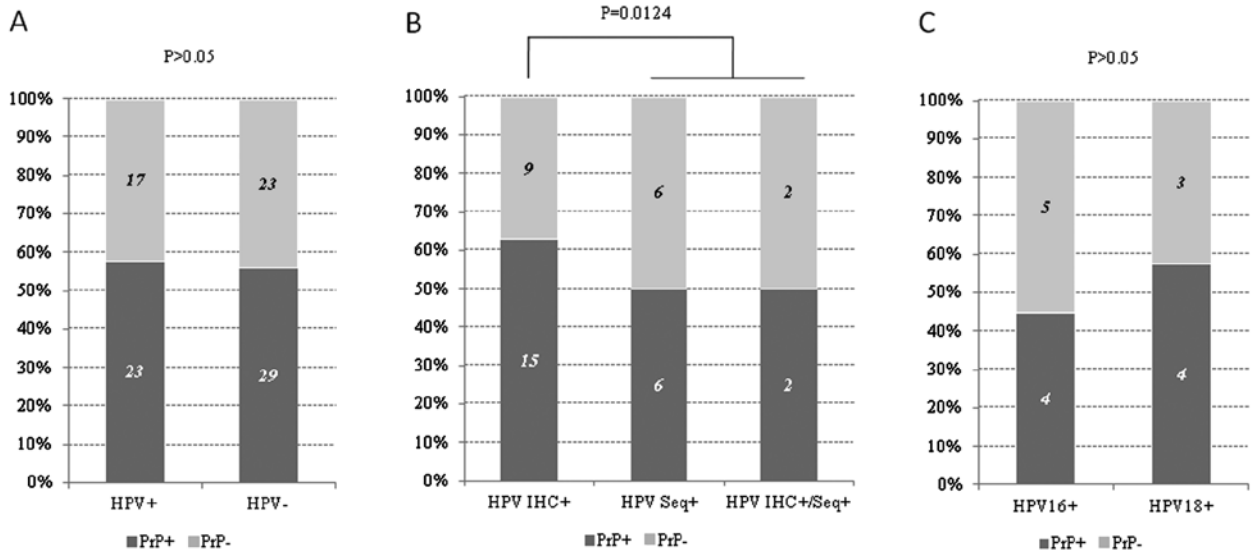

Figure 3. Correlation between PrP expression and HPV infection in the HNSCCs. (A) The percentage of PrP-positive staining in the patients with HPV positivity and with HPV negativity. (B) The percentage of PrP-positive staining in the patients with HPV protein positivity (HPV IHC+), HPV sequence positivity (HPV Seq+), both HPV sequence and protein positivity (HPV IHC+/Seq+). (C) The percentage of PrP-positive staining in the patients with HPV16 sequence positivity and HPV18 sequence positivity. PrP, prion protein; HNSCC, head and neck squamous cell carcinoma.

Correlation between PrP expression and HPV infection in HNSCCs. Among the 92 HNSCCs, HPV-related sequences and/or proteins were detectable in 40 cases. The positive rates of PrP immunostaining were 57.50% (23/40) and 55.76% (29/52) in the HPV-positive and -negative cases, respectively. No statistical difference in PrP expression was found between the HPV^+ and HPV^- groups (P>0.05) (Fig. 3A). Among the 40 HPV-positive HNSCCs, 12 were HPV sequence positive only, and 24 were HPV early (E6/E7) protein positive only, and 4 were HPV sequence and protein positive both (18). Obvious expression of PrP was detected in half of the patients in the HPV sequence positive (HPV Seq^+) and HPV sequence and protein positive (HPV IHC+/Seq^+) groups, but in 62.5% of the patients showing HPV protein positivity (HPV IHC^+), showing statistical difference (P<0.05) (Fig. 3B). Additionally, 4 out of 9 HPV16 sequence-positive cases and 4 out of 7 HPV18 sequence-positive cases were PrP-positive, without statistical significance (Fig. 3C). These data indicate that HPV infection does not influence the expression of PrP in malignant cells.

Discussion

In the present study, we screened the PrP expression in malignant tissues of HNSCCs with IHC. PrP-positive staining was observable in 55.43% of the tested HNSCCs. The positive rate and the intensity of PrP in the tumor tissues were closely associated with the pathological grade of the carcinomas. Meanwhile, the PrP-positive rate was correlated with the anatomic site and clinical degree of the HNSCCs. Additionally, the HNSCCs with p53-positivity in IHC and the Pro/Pro and Pro/Arg genotypes in SNP27 of p53 showed a higher probability for positive expression of PrP in their cells. PrP expression in the HNSCCs were not related with HPV infections.

Apart from its indispensible and unique role in human and animal prion diseases, a disparate significance of PrP in human malignant tumors has attracted great attention. Numerous lines of evidence support the potential involvement of PrP in different types of human cancers, in which PrP is overexpressed, such as colorectal (21), gastric (22,23) breast (24,25), pancreatic (26,27) and prostrate (28). In addition, PrP expression is observable in many tumor cell lines derived from the above malignant tumors or from other cancers, such as melanoma and hepatocarcinoma (16,27). However, the PrP status in cancer derived from squamous cells appears to be rarely described, except a report of oral squamous cell carcinomas in a Chinese journal (29). Our data in the present study strongly indicate a distinct involvement of PrP protein in the cancer biology of squamous cell carcinoma, at least HNSCCs.

Despite the diversities of the PrP-positive rates in different types of cancers from different studies, a close correlation between PrP expression and pathological and clinical grades of cancers has been well documented in almost all studies (16,27). The frequency of PrP expression was found to be ~40% in patients with pancreatic ductal cell adenocarcinoma, but only 13% in pancreatic intraepithelial neoplasia (PIN)-3 cases and none in PIN-2 and -1 cases (27). Higher PrP-positive rates and stronger PrP staining are also noted in breast and gastric cancers (24,30). More importantly, PrP expression appears to be associated with poor prognosis in pancreatic and breast cancers (24), which highlights a contributory role of PrP in tumor progression. In line with the above observations, our data confirmed that the positive rates and staining intensities of PrP correlate well with the progression of HNSCCs, particularly the pathological grade of the tumors, which emphasizes again a common phenomenon in different human malignant tumors. Further prospective analysis of the relationship between the survival times and PrP expression in HNSCCs will help to define the prognostic value of PrP IHC assays.

Disruption of the p53 network favours cell survival and tumor progression. Abnormalities of p53, either mutations in *TP53* or overexpression, are frequently observed in a number of human cancers, including HNSCCs, which is likely to be

associated with increased susceptibility to cancer development. Our data in the present study illustrated a positive correlation of PrP expression with p53- positive IHC but a negative correlation with mutated *TP53* in the HNSCCs. The exact biological meanings of such a pattern remain unclear. Recently, the abnormal accumulation of either WT or mutant p53 in the cytoplasm and nucleus were described in human cancers, such as neuroblastoma, retinoblastoma, breast and colon cancers (31-34). Notably, the aggregation of p53 could be amyloid-like, which highlights a possibility that p53 amyloid formation may participate in the malignant process (35).

SNP72 in *TP53* is able to influence the function of p53, in which *TP53* encoding Arg is more effective than that encoding Pro at inducing apoptosis and preventing cells from neoplastic development (7). The significance of the SNP72 polymorphism in association with the susceptibility of several types of cancers and the survival of cancer patients have been proposed (36). Our previous study found that HNSCC patients with a Pro polymorphism showed average younger onset-age and had higher percentages of strong p53-positivity in IHC (7). Coincidental with the clinical and pathological features of SNP72, we revealed that the patients with a Pro polymorphism in SNP72 had a significantly higher PrP-positive rate in the present study. This may indicate a possible cooperative effect of PrP and p53 in cancer biology.

HPV infections are frequently detected in HNSCCs (3,37). Among the tested 92 HNSCCs in the present study, 40 cases showed HPV positivity, either HPV sequences or proteins. We did not observe a notable difference in PrP expression between HPV-positive and HPV-negative groups, implying that HPV infection has little effect on PrP expression in HNSCCs. HPV infection in the 92 HNSCCs showed an anatomic increasing trend from inside to outside; more HPV-positive cases were noted in the lip and oropharynx than in the hypopharynx and larynx (18). However, PrP expression in the HNSCCs did not reveal anatomic-dependent alteration. As the limited numbers of other HNSCCs besides laryngeal cancers, a final conclusion of the relationship between HPV infection and PrP expression in HNSCCs requires large score assays.

Cellular PrP appears to be involved in many essential biological processes, such as cell adhesion, neurite outgrowth, synaptic transmission, oxidative stress, anti-apoptosis and neuroprotection, which are closely associated with cellular survival, proliferation and differentiation (38,39). Therefore, it is reasonable to speculate whether aberrant PrP function may contribute to tumorigenesis. However, numerous studies including ours demonstrated that the PrP proteins in cancer cells are an aglycosyl form of PrP or so-called 'pro-prion', which accumulate mainly in the cytoplasm but do not anchor on the cell membrane (40). In this situation, it is hard to simply attribute the role of PrP in tumorigenesis to its aberrantly enhanced biological effects. In fact, accumulation of aglycosyl PrP (not PrP^{Sc}) in the cytoplasm usually reduces the viability of many different types of cultured cell lines. Recently, many potential mechanisms have been proposed to explain the possible role of PrP in cancer cells, such as activation of the PI3K/Akt signaling pathway to upregulate cyclin D in gastric cancer cells (41), chemotherapy drug-induced PrP interaction with P-glycoprotein (P-gp; ATP-dependent drug-efflux pumps ABCB1) in drug-resistant MCF7 breast cancer cells, enhanced doxorubicin resistance via the ERK1/2 signaling pathway in MDA-MB-435 breast cancer cells (42), the fatal attraction between pro-PrP to filamin A in melanoma and pancreatic cancer cells (26). Nevertheless, a more comprehensive knowledge of PrPs may facilitate the understanding of the pathogenesis and the development of more effective tools for diagnosis, prognosis, therapy and prevention not only for TSEs, but also for a number of cancers.

Acknowledgements

The present study was supported by the Chinese National Natural Science Foundation Grants (81301429 and 81572048), the China Mega-Project for Infectious Disease (2011ZX10004-101 and 2012ZX10004215), and the SKLID Development Grant (2012SKLID102 and 2015SJLID503).

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