RESEARCH ARTICLE

P16INK4a Immunostaining but Lack of Human Papilloma Virus Type 16 in Cutaneous Squamous Cell Carcinoma and Basal Cell Carcinoma: a Report from West Iran

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Abstract

The tumor suppressor p16 is a biomarker for transforming human papilloma virus (HPV) infections that can lead to contradictory results in skin carcinomas. The aim of this study was to evaluate p16 expression and HPV-16 infection in the cutaneous basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). This case-control study was performed on paraffin blocks of BCCs and SCCs and normal skin (53, 36, and 44 cases, respectively), between 2006 to 2015. Initial sections for groups were stained with hematoxylin and eosin (H & E). Immunohistochemistry was performed for p16 expression and human papilloma virus type 16 (HPV-16) infection. Normal group was skin of mammoplasty specimens and normal skin tissue in the periphery of tumors. The mean age at diagnosis was 42.1, 61.7 and 71.4 years for normal, BCC and SCC groups, respectively. P16 positivity was more in SCC and BCC groups compared to normal group ($P<0.05$) and HPV was negative in all patients in three groups. Also, the mean age at diagnosis and P16-positivity were higher for the SCC group than the BCC group ($P<0.005$). In conclusion, in non-melanoma skin cancers (SCC and BCC), p16-positivity can be a prognostic factor but there is no correlation between HPV-16 and p16 in these tumors.

Keywords: Skin - P16 - HPV-16 - basal cell carcinoma - squamous cell carcinoma

Introduction

Non-melanoma skin cancer (NMSC) is the most common cancer affecting white-skinned individuals and the incidence is increasing worldwide (Lomas et al., 2012). Basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are the two major types of non-melanoma skin cancer (Payette et al., 2010). There are approximately 200,000 new cases of cutaneous SCC diagnosed each year in the US, with 1300 to 2300 deaths per year from metastatic disease (Hodges and Smoller,2002), and it is the second most common non-melanoma skin cancer (Shayanfar et al., 2016). BCC develops predominantly in sun-exposed skin in fair-skinned individuals prone to sunburn and it typically occurs in adults (Eshkoor et al., 2008). Recently, an increased expression of p16, a cell cycle regulatory tumor suppressor protein, has been demonstrated in cervical squamous neoplasms as a marker of malignancy. In contrast, studies performed in skin carcinomas led to contradictory results (Conscience et al., 2006). The tumor suppressor p16, encoded by the CDKN2/INK4a locus, has been reported mutated in ≥24% of SCC patients in oropharynx (Hodges and Smoller, 2002). Also, P16INK4a is a biomarker for transforming human papilloma virus (HPV) infections that can act as an adjunct to current cytological and histological assessment of cervical smears and biopsies, allowing the identification of those women with ambiguous results that require referral to colposcopy and potentially treatment (Tsoumpou et al., 2009). HPV is increasingly recognized as an important human carcinogen but its role in the aetiopathogenesis of BCC in immunocompetent individuals is unclear (Escutia et al., 2011). Also, the oncogenic role of HPV in cutaneous SCC has been suggested by several studies performed on immunosuppresed patients (Shayanfar et al., 2013).

The aim of this study is to evaluate p16 expression and HPV-16 infection in cutaneous SCC and BCC and also the correlation between these factors in the West of Iran.

Materials and Methods

This case-control study was performed on paraffin

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blocks of BCC and SCC patients with normal group (53, 36, and 44 cases, respectively), between of 2006 to 2015 in Special Clinic, Emam Khomeini and Emam Reza Hospitals in Kermanshah city, Iran. Formalin-fixed paraffin embedded tissue sections from BCC, SCC and normal skin biopsy specimens were cut into 4-micron thick sections and mounted on glass slides. Initial sections were stained with hematoxylin and eosin (H and E). The previous diagnosis of BCC and SCC was confirmed using criteria of basaloid proliferation, palisading, retraction artifact and mucin deposition for BCC and squamous morphology, presence of desmosomes and keratin pearls for SCC. The cases had the most of the criteria and in discrepancy between dermatopathologist, assistant and previous diagnosis, the case were censored. Normal group was chosen from skin of mammoplasty specimens and normal skin tissue in the periphery of tumors. Immunohistochemistry (IHC) method was done on unstained 4-micron sections in a two-step process involving first, the binding of a primary antibody to the antigen of interest and second, the detection of bound antibody by a chromogen according to manufacturer instructions. Mouse monoclonal antibody to HPV [Anti-Papillomavirus Type 16(HPV-16), clone: Cam vir-1, catalog No. AM362-5ME ready to use, BioGenex, CA94538] and mouse monoclonal antibody to P16 [Anti-P16/INK4A, clone: G175-405, catalog No AM540-5M ready to use, BioGenex, CA94538] were used as primary antibody. The first antibody stains nucleus in positive cells. The second antibody stains nucleus and/or cytoplasms in positive cells. Positive controls for HPV-16 were known cases of cervical cancer in liquid cytology and biopsy for P16 were known cases of SCC of skin. We didn't use control tissue from BioGenex. The positivity and percentage of it was estimated independently by dermatopathologist and assistant on a two-head microscope (Zeiss Axiostar plus) in magnification of 40 in all the sample and confirmed in magnifications of 100 and 400 and the mean percentage was applied. Cut-off value was considered positivity in 10% of cells (Figure 1). Histological subtypes of BCC as single or mixed forms were diagnosed on an agreement between dermatopathologist and assistant.

Statistical analysis

The data were analyzed by IBM SPSS version 19 (SPSS Inc., Chicago, IL, USA) that for correlation between the means was used T-test and other variables was used Chi-square test. The diagrams were plotted by Microsoft Office Excel 2007. P-value<0.05 was considered statistically significant.

Results

The mean age at diagnosis was 42.1, 61.7 and 71.4 years for normal, BCC and SCC groups, respectively (Table 1). There was a significant difference between BCC and SCC groups with normal group and also BCC group with SCC group (P<0.05). The mean age was higher in BCC and SCC groups compared to normal group and also was higher in SCC group compared to BCC group. Normal skin group was more from skin of women's mammoplasty

and therefore correlation between sex of normal group and BCC or SCC is incorrect. P16 positivity was more in SCC and BCC groups compared to normal group (P<0.05) and HPV-16 was negative in all patients in three groups, despite of cytology and tissue section positive controls. Due to small size of biopsies, definite characterization of well, moderate and poorly differentiated types of SCC was not possible. Infiltrative (35.8%), nodular (24.5%) and combination both (nodular plus infiltrative) (24.5%) had the most prevalence of type of pathology in BCC patients.

The correlation of variables in BCC and SCC groups with P16 positivity has been shown in Table 2. The mean age at diagnosis for SCC group and P16-positive was higher than BCC group (P=0.003).

The prevalence of type of pathology in BCC based on P16 has been shown in Table 3. The prevalence of infiltrative was more in both groups (P16-positive and
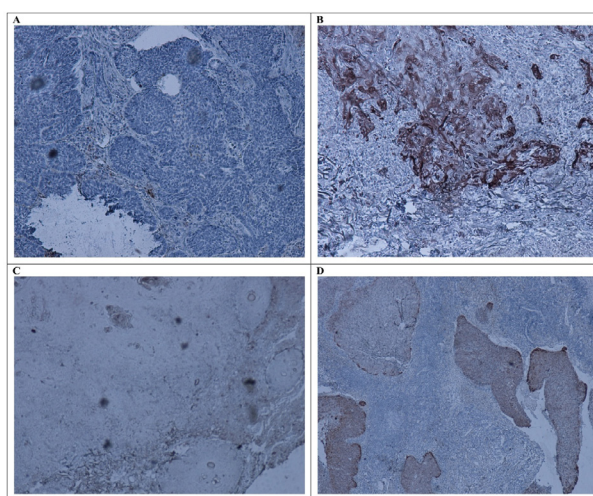

Figure 1. Immunohistochemistry Staining for Squamous Cell Carcinoma (SCC) and Basal Cell Carcinoma (BCC) (×100). (A) Negative staining and (B) positive staining for BCC, (C) negative staining and (D) positive staining for SCC

Table 1. Correlation of Variables in Three Groups (Normal, BCC and SCC)

Variables	Normal N=44	BCC N=53	SCC N=36
Age, years			
Mean±SD	42.1±16.9	61.7±14.6≠	71.4±13.7≠€
Range	20-81	21-89	32-92
Sex, male	7(15.9)¥	33(62.3)	23(63.9)
P16, positive	2(4.5)	42(79.2)≠	31(86.1)≠
HPV-16, positive	0	0	0
Type of Pathology in BCC			
Infiltrative		19(35.8)	
Nodular		13(24.5)	
Nodular and Infiltrative		13(24.5)	
Nodular and Micronodular		4(7.5)	
Micronodular		2(3.8)	
Nodular and keratotic		1(1.9)	
Superficial		1(1.9)	

Abbreviations: SCC, squamous cell carcinoma; BCC: basal cell carcinoma; HPV: human papilloma virus; SD, Standard Deviation. ≠A significant difference with normal group (P<0.05), € A significant difference with BCC group (P<0.05), ¥ More specimens were from skin of women's mammoplasty
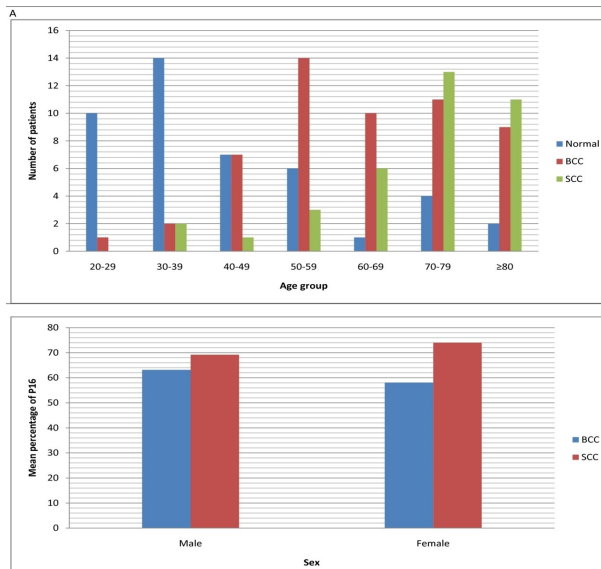
Figure 2. (A) Number of Patients Based on Age Groups for Normal, BCC and SCC and (B) Mean Percentage of P16 Positivity by Sex in BCC and SCC Groups

Table 2. Correlation of Variables in BCC and SCC Groups with P16-Positivity

Variables	BCC and P16-positive N=42	SCC and P16-positive N=31	P-value
Age, years			
Mean±SD	61.1±13.7	71.4±14.4	0.003*
Range	21-89	32-92	
Sex			0.426**
Male	25(59.5)	20(64.5)	
Female	17(40.5)	11(35.5)	
P16, %			0.222*
Mean±SD	30.7±15.5	35.8±19.8	
Range	10-60	10-80	

SCC, squamous cell carcinoma; BCC: basal cell carcinoma; SD, standard deviation; *T-test, **Chi-square test

Table 3. Prevalence of Type of Pathology in BCC Based on P16

Type of Pathology	P16-positive	P16-negative
Type of Pathology in BCC, n(%)		
Infiltrative	14(33.3)	5(45.4)
Nodular	9(21.4)	4(36.4)
Nodular and Infiltrative	12(28.6)	1(9.1)
Nodular and Micronodular	4(9.5)	0
Micronodular	2(4.8)	0
Nodular and Keratotic	0	1(9.1)
Superficial	1(2.4)	0

BCC: basal cell carcinoma

negative) compared to other types of pathology. There was no micronodular and superficial in P16-negative and there was no keratotic in P16-positive.

We divided age to seven groups and compared number of patients in three groups based on age group (Figure 2A). The most prevalence of BCC patients was in 50- 59 years, in SCC group was 70-79 years and in normal group was

30-39 years. The percentage of P16 in SCC group was more in female but in BCC group in male (Figure 2B).

Discussion

The incidence of non-melanoma skin cancer (SCC and BCC) is increasing every year (Payette et al., 2010). In a number of studies, p16 overexpression was observed in SCC patients (30-35%) (Alexander et al., 2012; Zhu et al., 2015), gastric carcinomas (0%)(Bashir et al., 2010), esophageal SCC (22%) (Kumar et al., 2015), oral SCC (86.66%) (Patil et al., 2014), head and neck SCCs (56%) (Klussmann et al., 2003), oropharyngeal SCC (78%) (Lewis et al., 2011), BCC (94.3%) (Paolini et al., 2011), and urothelial carcinoma with squamous differentiation (33%) (Alexander et al., 2012. One study (Conscience et al., 2006), reported that p16 overexpression was significantly observed in 58% of cutaneous carcinomas (SCC:60% and BCC:50%) versus 0% of normal human skin. In another study (Santos et al., 2004), p16 was positive in 60% of BCCs and 91% in keratinizing SCC.

A total of 142 samples from 70 BCC cases (superficial BCC and nodular BCC) and 72 controls were analyzed by a degenerated nested PCR technique. There were 31 HPV DNA-positive samples. HPV was detected more frequently in cases (25.7%) than in controls (18.1%) but differences were not statistically significant (Escutia et al., 2011), while 60-70% of oropharynx tumors may be HPV-positive, only 10 to 19% of tumors of the oral cavity, larynx and hypopharynx appear to have HPV infection (Bixofis et al., 2014). In p16-positive oropharyngeal SCC patients, 74% were positive for HPV by in situ hybridization (Lewis et al., 2011). Recent analyses of head and neck SCCs revealed frequent infections by oncogenic HPV-16 in tonsillar carcinomas. In 88.9% of HPV-positive carcinomas diffuse p16 expression was observed (Klussmann et al., 2003). Strong p16 overexpression has been observed by immunohistochemical analysis in SCC of the cervix infected by HPVs (Sano et al.,1998). In cutaneous SCC, HPV status is also associated with p16 expression (Tufaro et al., 2011). HPV-DNA and protein were not detected in 42 cases of SCC of the urinary bladder (0%) or 27 cases of urothelial carcinoma with squamous differentiation (0%). There was no correlation between p16 expression and the presence of HPV infection in SCC of the bladder or urothelial carcinoma with squamous differentiation (Alexander et al., 2012). P16INK4A IHC seems to be a superior marker for the detection of HPV-associated penile SCC compared to in-situ hybridization (Aumayr et al., 2013). It was found that HPV-16 prevalence in tongue cancers was 51.2% and HPV-16 being present in 85.2% of p16-positive cases. Also, another significant finding was a very poor concordance between p16 expression and HPV infection and shows that p16 expression should possibly not be used as a surrogate marker for HPV infection in tongue cancers (Ramshankar et al., 2014). In one study (Mokhtari et al., 2009), was found a significant relationship between BCC and HPV by IHC method.

However, it does not guarantee that a tumor is HPV-positive as other pathways may lead to p16 overexpression.

Mazaher Ramezani et al

In fact, studies have shown that approximately 15% to 20% of p16-positive oropharyngeal SCC cases are HPV-negative. However, the features of such tumors and their clinical behavior are unknown (Smeets et al., 2007). These data indicate that p16 is a technically simple immunohistological marker, applicable for routine pathological histology, and its prognostic value for survival is fully equivalent to HPV-DNA detection. In this study, p16 positivity by IHC method was 86.1% in cutaneous SCC and 79.2% in cutaneous BCC patients compared to 4.5% in normal skin group that there was significant difference between normal group and two other groups. Also, HPV-16 infection by IHC method was negative in three groups.

In conclusion, In non-melanoma skin cancers (SCC and BCC), p16-positive can be a prognostic factor but there is no the correlation between HPV-16 and p16 in these tumors.

Acknowledgements

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