Human papillomavirus infection in head and neck cancer: The role of the secretory leukocyte protease inhibitor

MARKUS HOFFMANN1, ELGAR S. QUABIUS1,2*, SILKE TRIBIUS3, LENA HEBEBRAND1, TIBOR GÖRÖGH1, GORDANA HALEC4, TOMAS KAHN5, JÜRGEN HEDDERICH6, CHRISTOPH RÖCKEN7, JOCHEN HAAG7, TIM WATERBOER8, MARKUS SCHMITT9, ANNA R. GIULIANO8 and W. MARTIN KAST9

1Department of Otorhinolaryngology, Head and Neck Surgery; 2Institute of Immunology, Christian-Albrechts University Kiel, D-24105 Kiel; 3Department of Radiation Oncology, University Medical Center Hamburg-Eppendorf, D-20246 Hamburg; 4German Cancer Research Center (DKFZ), Infection and Cancer Program (F020), D-69120 Heidelberg; 5Expert Team Life Sciences, Deutsche Bank AG, D-60311 Frankfurt; 6Institute for Medical Informatics and Statistics, University Hospital of Schleswig-Holstein, Campus Kiel, D-24015 Kiel; 7Institute for Pathology, Christian-Albrechts-University Kiel, D-24105 Kiel, Germany; 8Center for Infection and Cancer Research, and the Department of Cancer Epidemiology, H. Lee Moffitt Cancer Center, Tampa, FL 33612; 9Departments of Molecular Microbiology and Immunology, and Obstetrics and Gynecology, Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA 90033, USA

Received November 20, 2012; Accepted December 17, 2012

DOI: 10.3892/or.2013.2327

Abstract. We previously showed that secretory leukocyte protease inhibitor (SLPI) gene and protein expression is significantly lower in metastatic versus non-metastatic head and neck squamous cell carcinoma (HNSCC). However, we did not assess the human papillomavirus (HPV) status of these cases. Since SLPI plays a role in HIV and herpes simplex virus (HSV) infections, we hypothesized that SLPI may be involved in HPV-infected HNSCC. In HNSCC tissue (n=54), HPV DNA was determined and correlated with SLPI expression. Additionally, to investigate a possible role of smoking on SLPI expression in clinically normal mucosa, 19 patients treated for non-malignant diseases (non-HNSCC) were analyzed for SLPI expression and correlated with smoking habits. In HNSCC patients, SLPI expression showed a significant inverse correlation with HPV status. In patients with moderate/strong SLPI expression (n=19), 10.5% were HPV-positive. By contrast, patients with absent/weak SLPI expression (n=35), 45.7% were HPV-positive. Low SLPI expression was correlated with metastasis (P=0.003) independent of HPV status. HPV-positivity was clearly associated with lymph node status (81.3% N1-3 cases). In smoking non-HNSCC patients (n=7), 42.9% showed absent/weak and 57.1% moderate/strong SLPI staining. In non-smoking non-HNSCC patients (n=10) 83.3% showed absent/weak and 16.7% moderate/strong SLPI expression. For the first time, a correlation between SLPI downregulation and HPV infection was demonstrated, suggesting that high levels of SLPI, possibly induced by environmental factors such as tobacco smoking, correlate with protective effects against HPV infection. SLPI may be a potential biomarker identifying head and neck cancer patients not at risk of developing metastases (SLPI-positive), and those at risk to be infected by HPV (SLPI-negative) and likely to develop metastases.

Introduction

We previously demonstrated that secretory leukocyte protease inhibitor (SLPI) is significantly associated with head and neck squamous cell carcinoma (HNSCC) (1). Although we did not assess human papillomavirus (HPV) status, the viral etiological agent in a subset of HNSCC (2-5), we, and others, repeatedly showed a correlation between HPV infection and metastases of these tumors (6-8). Additionally, SLPI has been associated with other viral infections and it has been demonstrated that the prevalence of oral HIV is reduced in cases of elevated SLPI expression (reviewed in ref. 9). By contrast, herpes simplex virus (HSV) downregulates SLPI levels in a cell culture model (10). Therefore, we evaluated the association between HPV status and SLPI expression in a series of tumors using multiple markers of HPV infection across multiple anatomical sites within the head and neck and correlated this with occurrence of metastases.

The SLPI protein, also known as antileukoprotease, is a 11.7-kDa (107 amino acids) non-glycosylated kazal-type serine protease inhibitor of neutrophil elastase, cathepsin G, chymotrypsin and trypsin (11). It is produced by different cell
types including breast, lung, endometrium, ovary, salivary glands and by various host inflammatory and immune cells such as macrophages, neutrophils and B lymphocytes (12-14). In particular, the role of SLPI as a potent inhibitor of neutrophil elastase appears to be an important factor resulting in the protection of the mucosa and skin against proteolysis (13,15).

In HNSCC, two possible types of carcinogenesis are discussed: i) an HPV-driven carcinogenesis, leading to biologically more aggressive tumors including early metastatic spread into the locoregional lymph nodes, but with comparatively better survival rates of the patients (8,16); and ii) a tobacco/alcohol-driven carcinogenesis, leading to delayed onset of disease with a time lapse of approximately 10 years (17). However, recent data suggest a synergism between HPV and smoking (16).

Moreover, a recent report showed a positive correlation between cigarette smoke and SLPI-expression in a rat model (18). This finding motivated us to further study SLPI expression in healthy mucosal tissue of non-HNSCC patients and to correlate SLPI expression levels with smoking habits.

Materials and methods

Patients, tissues specimens, DNA and RNA extraction. From 2004 to 2009, tissue samples of histopathologically confirmed HNSCC were obtained from 54 patients (42 male and 12 female; range, 43-76 years; median, 58 years) during surgery at the Department of Otorhinolaryngology, Head and Neck Surgery at the Christian-Albrechts-University Kiel, Germany. All samples were obtained following informed consent approved by the local Ethics Committee (D 438/10). Tissue samples were treated as follows: one section of the tumor tissue was snap-frozen in liquid nitrogen and stored at -80°C for further analysis. The remaining section of each tumor was processed for routine histopathology. DNA was extracted from 25 mg frozen tissue samples, as previously described (20,19). Total RNA was extracted using peqGOLD TriFast reagent (PeqLab, Erlangen, Germany) according to the manufacturer's protocol.

The anatomical location of the primary tumors was: tonsils (n=20); palatine tonsil (n=14), lingual tonsil (n=6), larynx (n=22), tongue (n=8), and soft palate (n=4). SCC of the tongue (anatomical localization of the oral cavity) and SCC of the soft palate (per definition anatomical localization of the oropharynx) were investigated in one subgroup (n=12) since the biological behavior of soft palate SCC is more comparable to oral cavity SCC than to tonsillar SCC (per definition anatomical localization of the oropharynx). Additional clinicopathological characteristics and tobacco smoking habits are presented in Table I.

We also obtained tissue samples from clinically normal mucosa of the aerodigestive tract from 19 patients (12 male and 7 female; range, 2-63 years; median, 43 years) who were treated for non-malignant diseases at our Department. Additional clinicopathological characteristics and tobacco smoking habits are presented in Table II.

Detection of HPV DNA. Two different PCR-based detection methods for HPV DNA were applied to increase precision in validity and reliability of HPV DNA diagnostics: multiplex HPV genotyping (MPG) assay and HPV(73) 3.5 LCD-Array, as previously described (20-22; Table I).
Table I. Demographic and clinical characteristics of the patients and results of the tumors investigated.

| Age/gender (years) | Tumor site | TNM     | HPV DNA\(^b\) | E6*I mRNA\(^b\) | Viral activity\(^c\) | SLPI\(^d\) | Smoking habits\(^e\) |
|-------------------|------------|---------|----------------|-----------------|-----------------------|-----------|----------------------|
| 1 47/M            | Lingual    | T4N2bM0 | -              | ++              | >40 py                |           |  |
| 2 67/F            | Palatine   | T2N0M0  | HPV16          | Active          | +                     |           | never               |
| 3 61/M            | Lingual    | T3N2bM0 | HPV16          | Active          | ++ na                 |           | na                  |
| 4 61/M            | Palatine   | T4N2bM0 | -              | -               | >40 py                |           | na                  |
| 5 63/M            | Palatine   | T1N2bM0 | HPV16          | Active          | - na                  |           | na                  |
| 6 53/F            | Palatine   | T2N1M0  | HPV16          | Active          | +++ >20 py            |           | na                  |
| 7 64/M            | Palatine   | T1N1M0  | HPV16          | Active          | - >20 py              |           | na                  |
| 8 70/F            | Palatine   | T3N1M0  | HPV16          | Active          | + never               |           | na                  |
| 9 60/M            | Lingual    | T3N2bM0 | HPV16          | Active          | + na                  |           | na                  |
| 10 51/M           | Lingual    | T2N1M0  | HPV16          | Active          | + >20 py              |           | na                  |
| 11 76/M           | Lingual    | T4N0M0  |                | ++              | >20 py                |           | na                  |
| 12 53/M           | Palatine   | T1N2aM0 |                | ++              | <20 py                |           | na                  |
| 13 52/M           | Palatine   | T1N2bM0 | HPV16          | Active          | + >20 py              |           | na                  |
| 14 54/M           | Palatine   | T3N0M0  |                | ++              | >40 py                |           | na                  |
| 15 59/M           | Palatine   | T2N0M0  |                | +               | >40 py                |           | na                  |
| 16 47/M           | Palatine   | T4N2bM0 |                | +               | >20 py                |           | na                  |
| 17 67/F           | Palatine   | T3N2cM0 | HPV16          | Active          | - never               |           | na                  |
| 18 72/M           | Lingual    | T4N2bM0 |                | +               | na                    |           | na                  |
| 19 63/M           | Palatine   | T4N2cM0 | HPV18          | Active\(^f\)   | - >40 py              |           | na                  |
| 20 68/M           | Palatine   | T2N0M0  | HPV16/18       | Active          | + >20 py              |           | na                  |
| 21 54/M           | Larynx     | T3N0M0  |                | +++             | >40 py                |           | na                  |
| 22 53/F           | Larynx     | T4N0M1  |                | -               | >20 py                |           | na                  |
| 23 72/M           | Larynx     | T3N0M0  | HPV16          | Active          | - >20 py              |           | na                  |
| 24 52/F           | Larynx     | T2N2cM0 | HPV16          | Active          | - na                  |           | na                  |
| 25 69/M           | Larynx     | T2N0M0  |                | ++              | na                    |           | na                  |
| 26 70/M           | Larynx     | T4aN1M0 | HPV16          | Inactive\(^f\) | + >20 py              |           | na                  |
| 27 61/M           | Larynx     | T4N2bM0 |                | -               | >40 py                |           | na                  |
| 28 55/M           | Larynx     | T3N2cM0 |                | +               | >20 py                |           | na                  |
| 29 73/F           | Larynx     | T3N2cM0 |                | +               | never                 |           | na                  |
| 30 48/F           | Larynx     | T3N0M0  | HPV16          | +               | >20 py                |           | na                  |
| 31 58/M           | Larynx     | T3N2cM0 |                | +               | >40 py                |           | na                  |
| 32 73/M           | Larynx     | T3N0M0  |                | -               | >40 py                |           | na                  |
| 33 54/F           | Larynx     | T4N2cM0 | HPV16          | Inactive        | - na                  |           | na                  |
| 34 75/M           | Larynx     | T4aN0M0 |                | +++             | >20 py                |           | na                  |
| 35 54/M           | Larynx     | T3N1M0  |                | +               | <20 py                |           | na                  |
| 36 55/F           | Larynx     | T3N0M0  |                | ++              | >40 py                |           | na                  |
| 37 58/M           | Larynx     | T4N1M0  |                | +               | <20 py                |           | na                  |
| 38 56/M           | Larynx     | T3N0M0  |                | +++             | >40 py                |           | na                  |
| 39 69/M           | Larynx     | T1N2cM1 |                | +               | >40 py                |           | na                  |
| 40 73/M           | Larynx     | T3N0M0  |                | ++              | >40 py                |           | na                  |
| 41 58/F           | Larynx     | T4N2cM0 |                | +               | >20 py                |           | na                  |
| 42 64/M           | Larynx     | T3N3M0  | HPV16          | ++              | >20 py                |           | na                  |
| 43 56/M           | Tongue     | T4N3M0  |                | ++              | >40 py                |           | na                  |
| 44 57/M           | Tongue     | T4aN0M0 | HPV16          | ++              | >40 py                |           | na                  |
| 45 53/M           | Tongue     | T4N2cMx |                | +++             | na                    |           | na                  |
| 46 43/M           | Tongue     | T4N2cM0 |                | +               | >40 py                |           | na                  |
| 47 57/M           | Tongue     | T3N2bM0 | HPV16          | Active          | - never               |           | na                  |
With HPV16. From the 12 carcinomas of the tongue and the soft palate, 16.7% were HPV16-positive. The prevalence was significantly higher in the tonsillar tumors (P=0.009).
17 cases, of which 14 could be assigned to HPV DNA-positive cases. The remaining 3 cases were classified as HPV-negative due to lack of HPV DNA-positivity according to the applied algorithm. However, considering HPV RNA expression as more important for an active HPV infection than existence of HPV DNA, these 3 cases would need to be classified as cases with active HPV infection. Only 1 out of the 17 HPV DNA positive cases with available RNA did not show detectable HPV mRNA. This case was classified as inactive infection, whereas the 14 HPV DNA positive cases with detectable levels of viral mRNA were classified as active HPV infections.

In 3 HPV DNA-positive cases, RNA was not available for analysis. In these cases, p16INK4A immunohistochemistry was performed. Due to strong p16INK4A staining in 2 cases, these were classified as active HPV infections. Collectively, 16/18 cases with HPV DNA were finally classified as active HPV infections.

**Immunohistochemistry for SLPI**

**SLPI correlation with HPV DNA status.** In 25.9% of 54 cases, there was no SLPI staining detectable. Irrespective of the HPV status, absent, weak, moderate/strong staining was detected in 38.8, 24.1 and 11.1% of SLPI-positive cases, respectively (Table III). The cases with absent/weak and the cases with moderate/strong SLPI staining were combined for further correlation analysis: out of 19 cases with moderate/strong SLPI reactivity, 89.5% were HPV-negative and 10.5% were HPV-positive; 54.3% cases with absent/weak SLPI expression (n=35) were HPV-negative, whereas 45.7% were HPV-positive. Thus, these data suggest that low SLPI expression is associated with HPV infection, whereas elevated SLPI expression appears to prevent such an infection. In the case of classifying the 3 cases with HPV RNA but no HPV DNA as active HPV infections, this correlation would be even stronger, since 2 of these cases showed absent/weak SLPI expression.

**Lymph node-(N) status depending on SLPI expression, HPV infection status, alone and in combination.** The distribution of the N0 vs. the N1-3 cases among the group of absent/weak and moderate/strong was 20 vs. 80%, and 63.2 vs. 36.8% (P=0.003), respectively. Elevated SLPI was associated with lower metastasis whereas absent/weak abundance of SLPI was associated with increased tumor burden of the neck. The distribution of patients with (n=22) and without (n=16) metastasis among the HPV-negative cases was similar. However, out of the HPV-positive cases (n=16), 13 developed metastases. The correlation between the HPV status, SLPI expression and the N-status was highly significant (P=0.003), despite the relatively small number of cases in each group (Table IV). Independent of HPV status, reduced SLPI levels were associated with tumor masses in the neck. However, the association between higher SLPI and lower prevalence of metastasis was only found in the absence of an HPV infection.

**SLPI expression in clinically normal mucosa.** SLPI immunohistochemistry of clinically normal mucosa obtained from 7 non-HNSCC patients with history of tobacco smoking showed absent/weak and moderate/strong staining in 42.9 and 57.1% cases, respectively. Patients without a history of tobacco smoking (n=12) showed absent/weak and moderate/strong staining patterns in 83.3 and 16.7% cases each (Table II). Although the absolute differences did not reach statistical significance (P=0.085) due to the small sample size the trend indicates a strong correlation between smoking habits and SLPI expression (P<0.0001).

**Discussion**

To our knowledge, this is the first report showing a correlation between: i) SLPI expression and HPV infection; ii) SLPI expression, HPV infection and lymph nodal disease in head and neck cancer; and iii) SLPI expression and tobacco consumption habits in clinically normal mucosa of non-HNSCC patients.

The data presented here demonstrate that high SLPI expression is correlated with reduced prevalence of HPV infection in the investigated tumor specimens of HNSCC, while the HPV-positive cases predominate in the group of absent to low SLPI expression. Therefore, even in the presence of HPV DNA, SLPI expression may prevent an HPV infection of the upper aerodigestive tract. These findings are in agreement with the current available literature regarding the role of SLPI in oral HIV infections. For example, McNeely et al (24) suggested that SLPI inhibits HIV infections by blocking HIV binding to the host cells. In agreement with the effects of SLPI

| Table III. Distribution of SLPI expression levels among HPV DNA-positive and -negative cases. |
|---------------------------------|---|---|---|---|
|                                  | SLPI             |
|                                 | -   | +  | ++/+++ | Total |
| HPV-positive                    | 9   | 7  | 2      | 18   |
| HPV-negative                    | 5   | 14 | 17     | 36   |
| Total                           | 14  | 21 | 19     | 54   |

SLPI antibody reactivity was scored on a semi-quantitative scale, according to Cordes et al (1). The correlation shown here is statistically significant (P=0.005). When analyzing the SLPI negative (-) cases together with the cases with weak (+) SLPI expression against cases with moderate to strong (++/++++) expression the P=0.01. SLPI, secretory leukocyte protease inhibitor.

| Table IV. N-status depending on HPV infection status and SLPI expression level. |
|---------------------------------|---|---|---|---|
|                                  | N-status |
|                                | 0  | 1-3 | Total |
| HPV-negative and SLPI-/+         | 4  | 15 | 19   |
| HPV-negative and SLPI ++/+++     | 12 | 5  | 17   |
| HPV-positive and SLPI -/+        | 3  | 13 | 16   |
| HPV-positive and SLPI ++/+++     | 0  | 2  | 2    |
| Total                           | 19 | 35 | 54   |

The correlation is statistically significant (P=0.003). SLPI, secretory leukocyte protease inhibitor.
on HIV. Woodham *et al* recently showed that SLPI incubation of epithelial cells reduces HPV entry into these cells in a dose-dependent manner. Furthermore, SLPI was shown to bind to the Annexin A2 heterotetramer (A2t). In turn, A2t is associated with HPV insertion into and infection of epithelial cells (25). Therefore, our data support the theory of the existence of a cellular receptor for HPV and the role of SLPI as a competing agent for such receptor binding sites.

In addition, Wahl *et al* (26) suggested that SLPI mediates its anti-viral activity by affecting the host cells rather than the virus itself. In line with these findings, we found only 2 cases with high SLPI expression that were also HPV-positive. However, it remains unclear why these 2 cases had a higher lymph nodal status, suggesting that in cases with HPV infection, SLPI might lose its protective role against metastasis. Such a protective role of SLPI has previously been described (27,28), and has been assigned to the theory that SLPI affects the invasive activity of cancer cells by inhibiting enzymes promoting cancer invasion and progression. It is postulated that the absence or repression of the SLPI-antileukokoprotease function, as we described here in metastatic tumor specimens, promotes spreading of the tumor by enabling degradation of surrounding tissues by proteases, secreted from the tumors.

We previously reported that significant elevation of SLPI is associated with non-metastasized HNSCC (1), a phenomenon which could be confirmed on mRNA as well as protein levels. Similarly, high statistical significance was again obtained when stratifying absent/weak and moderate/strong SLPI expression against tumor burden of the neck, using the data presented here. In contrast to our own previously published data on SLPI in metastasized and non-metastasized HNSCC showing no correlation between the expression of SLPI and the degree of tumor differentiation (1), other researchers (29,30) demonstrated such a correlation in human epidermal tumors, leading to the hypothesis that SLPI protein levels represent a negative surrogate marker for tumor progression, thus corroborating our present data regarding SLPI expression and metastasis in HNSCC. However, the exact mode of action of SLPI in carcinogenesis and metastasis remains unclear.

The role of SLPI as a potent inhibitor of neutrophil elastase appears to be an important factor resulting in the protection of the mucosa and skin against proteolysis. In addition, Chan *et al* (18) showed that total neutrophil elastase concentration and activity as well as SLPI activity was increased following exposure to cigarette smoke. This finding led us to investigate the role of SLPI in healthy mucosa of non-HNSCC patients and to correlate SLPI expression with smoking habits. In accordance with the data obtained in a rat model (18), we indeed showed that moderate/strong SLPI expression was mostly found in patients with smoking habits. In HNSCC, it is well documented that HPV-driven carcinogenesis occurs in younger patients when compared to patients with alcohol/tobacco-driven carcinogenesis (4,17). According to our data, it can be assumed that smoking-induced SLPI expression prevents HPV infection, resulting in delayed agent-dependent cancer with poorer prognosis.

In conclusion, in the present study we identified for the first time a statistically significant inverse correlation between HPV infection and SLPI expression levels, suggesting that reduced expression of SLPI facilitates HPV infection. The previously described correlation between SLPI reduction, HPV infection, and metastasis was confirmed. In addition, we provided preliminary evidence that tobacco smoking is associated with SLPI expression.

**Acknowledgements**

The authors thank Professor E. Schwarz (German Cancer Research Center) and Dr Diane Da Silva (USC) for their critical and constructive reading of the manuscript, Dr Christian Cordes, Department of Otorhinolaryngology, Head and Neck Surgery, Christian-Albrechts-University (CAU) Kiel, Germany, for his skillful assistance evaluating the results of SLPI immunohistochemistry, and Anne-Marie Roen (CAU) for her skillful technical assistance. This study was supported by NIH grant ROI CA74397-13 (to W.M.K.) and the Dekanat of the Medical Faculty of the Christian-Albrechts-University, Kiel, Germany (to M.H.).

**References**

1. Cordes C, Hasler R, Werner C, Gorogth T, *et al*: The level of secretory leukocyte protease inhibitor is decreased in metastatic head and neck squamous cell carcinoma. Int J Oncol 39: 185-191, 2011.
2. Hoffmann M, Ihloff AS, Gorogth T, *et al*: p16(INK4a) overexpression predicts translational active human papillomavirus infection in tonsillar cancer. Int J Cancer 127: 1595-1602, 2010.
3. Kreimer AR, Clifford GM, Boyle P, *et al*: Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev 14: 467-475, 2005.
4. Smith EM, Ritchie JM, Summersgill KF, *et al*: Age, sexual behavior and human papillomavirus infection in oral cavity and oropharyngeal carcinomas. Int J Cancer 108: 766-772, 2004.
5. Termine N, Panzarella V, Falaschini S, *et al*: HPV in oral squamous cell carcinoma vs head and neck squamous cell carcinoma biopsies: a meta-analysis (1988-2007). Ann Oncol 19: 1691-1690, 2008.
6. Hafkamp HC, Manni JJ, Haesevoets A, *et al*: Marked differences in survival rate between smokers and nonsmokers with HPV 16-associated tonsillar carcinomas. Int J Cancer 122: 2656-2664, 2008.
7. Hoffmann M, Gottschlich S, Gorogth T, *et al*: Human papillomavirus-infections in lymph node metastases of head and neck cancers. Acta Otolaryngol 125: 415-421, 2005.
8. Hoffmann M, Gorogth T, Gottschlich S, *et al*: Human papillomavirus-infections in head and neck cancer: 8 year-survival-analysis of 73 patients. Cancer Lett 218: 199-206, 2005.
9. Drahnik AG, Henrick BM and Rosenthal KL: War and peace between WAP and HIV: role of SLPI, trappin-2, elafin and ps20 in susceptibility to HIV infection. Biochem Soc Trans 39: 1427-1432, 2011.
10. Panki E, Wilson SS, Mesquita PM, *et al*: Herpes simplex virus downregulates secretory leukocyte protease inhibitor: a novel immune evasion mechanism. J Virol 82: 9337-9344, 2008.
11. Boudier C, Cadene M and Bieth JG: Inhibition of neutrophil cathepsin G by oxidized mucus proteinase inhibitor. Effect of heparin. Biochemistry 29: 8451-8457, 1999.
12. Abe T, Kohayashi N, Yoshimura K, *et al*: Expression of the secretory leukokrotease inhibitor gene in epithelial cells. J Clin Invest 87: 2207-2215, 1991.
13. Franken C, Meijer CJ and Dijkman JH: Tissue distribution of antileukokrotease and lysozyme in humans. J Histochem Cytochem 37: 493-498, 1989.
14. Jin FY, Nathan C, Radzioch D and Ding A: Secretory leukocyte protease inhibitor: a macrophage product induced by and antagonistic to bacterial lipopolysaccharide. Cell 7: 417-426, 1997.
15. Hafkamp HC, Manni JJ, Haesevoets A, *et al*: Human papillomavirus-infections in lymph node metastases of head and neck cancers. Acta Otolaryngol 125: 415-421, 2005.
16. Ang KK, Harris J, Wheeler R, *et al*: Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 363: 24-35, 2010.
17. Cole L, Polfus L and Peters ES: Examining the incidence of human papillomavirus-associated head and neck cancers by race and ethnicity in the U.S., 1995-2005. PLoS One 7: e32657, 2012.
18. Chan KH, Chan SC, Yeung SC, et al: Inhibitory effect of Chinese green tea on cigarette smoke-induced up-regulation of airway neutrophil elastase and matrix metalloproteinase-12 via antioxidant activity. Free Radic Res 46: 1123-1129, 2012.

19. Hoffmann M, Tribius S, Quabius ES, et al: HPV DNA, E6(*) I-mRNA expression and p16(INK4A) immunohistochemistry in head and neck cancer - how valid is p16(INK4A) as surrogate marker? Cancer Lett 323: 88-96, 2012.

20. Remmerbach TW, Brinckmann UG, Hemprich A, et al: PCR detection of human papillomavirus of the mucosa: comparison between MY09/11 and GP5+/6+ primer sets. J Clin Virol 30: 302-308, 2004.

21. Schmitt M, Bravo IG, Snijders PJ, et al: Bead-based multiplex genotyping of human papillomaviruses. J Clin Microbiol 44: 504-512, 2006.

22. Schmitt M, Dondog B, Waterboer T, et al: Homogeneous amplification of genital human alpha papillomaviruses by PCR using novel broad-spectrum GP5+ and GP6+ primers. J Clin Microbiol 46: 1050-1059, 2008.

23. Halec G, Schmitt M, Dondog B, et al: Biological activity of probable/possible high-risk human papillomavirus types in cervical cancer. Int J Cancer 132: 63-71, 2013.

24. McNeely TB, Shugars DC, Rosendahl M, et al: Inhibition of human immunodeficiency virus type 1 infectivity by secretory leukocyte protease inhibitor occurs prior to viral reverse transcription. Blood 90: 1141-1149, 1997.

25. Woodham AW, Da Silva DM, Skeate JG, et al: The S100A10 subunit of the annexin A2 heterotetramer facilitates L2-mediated human papillomavirus infection. PLoS One 7: e43519, 2012.

26. Wahl SM, McNeely TB, Janoff EN, et al: Secretory leukocyte protease inhibitor (SLPI) in mucosal fluids inhibits HIV-1. Oral Dis 3 (Suppl 1): S64-S69, 1997.

27. Sun Z and Yang P: Role of imbalance between neutrophil elastase and alpha 1-antitrypsin in cancer development and progression. Lancet Oncol 5: 182-190, 2004.

28. Nakamura K, Takamoto N, Hongo A, et al: Secretory leukoprotease inhibitor inhibits cell growth through apoptotic pathway on ovarian cancer. Oncol Rep 19: 1085-1091, 2008.

29. Alkemade HA, van Vlijmen-Willems IM, van Haelst UJ, et al: Demonstration of skin-derived antileukoproteinase (SKALP) and its target enzyme human leukocyte elastase in squamous cell carcinoma. J Pathol 174: 121-129, 1994.

30. Westin U, Nyström M, Ljungrantz I, et al: The presence of elafin, SLPI, IL1-RA and STNFalpha RI in head and neck squamous cell carcinomas and their relation to the degree of tumour differentiation. Mediators Inflamm 11: 7-12, 2002.