Expression of p16 in Sinonasal Undifferentiated Carcinoma (SNUC) Without Associated Human Papillomavirus (HPV)

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Abstract Sinonasal undifferentiated carcinoma (SNUC) is an uncommon and highly aggressive neoplasm of the paranasal sinuses and nasal cavity. Its undifferentiated histologic appearance often requires immunohistochemical studies to distinguish it from other high-grade neoplasms. Due to the rarity of SNUC, its immunohistochemical staining profile has been incompletely characterized, and little work has been done on its expression of the markers for human papillomavirus (HPV). Our objective is to expand our knowledge of its immunophenotype and its association with HPV in order to define markers with mechanistic potential in the disease process, or of possible therapeutic importance. A total of five patients (one woman and four men) with SNUC, ranging in age from 26 to 75 years (mean 56.8 years) were compared to five patients (five men) with poorly differentiated squamous cell carcinoma (PDSCC), ranging in age from 53 to 75 years (mean 62.2 years). PDSCC was chosen as a control, given its well-reported immunohistochemical profile and negativity

for HPV markers. The immunohistochemical panel included: CK7, CK19, EMA, NSE, chromogranin, p53, CK5/6, p63, CK14, S100, HMB-45, desmin, muscle specific actin, and CD45. Additionally, tests for p16, EBV, and HPV (subtypes 6, 11 16, 18) were performed. The diagnosis of SNUC was confirmed in all cases by histology and immunohistochemical stains. An interesting finding of strong diffuse positivity for p16 was noted in all SNUC cases, compared to only two of five PDSCC that were positive for p16. HPV DNA was not detected in any SNUC cases or any cases of PDSCC. All SNUC cases demonstrated over expression of p16 in the absence of HPV DNA expression. This may represent residual epithelial p16 staining, which is normally present in the sinonasal tract. Due to the rarity of SNUC, more cases will need to be evaluated to confirm the absence of HPV DNA.

Keywords Human papillomavirus (HPV) · Sinonasal undifferentiated carcinoma (SNUC) · Epstein Barr virus (EBV) · Sinonasal carcinoma · Poorly differentiated squamous cell carcinoma

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Introduction

The most commonly encountered malignant neoplasms of the nasopharynx are squamous cell carcinomas. Although less frequent, a more aggressive group of non-epithelial malignant neoplasms exists in the nasopharynx and sinonasal tract that must be recognized. The group consists of “round blue cell neoplasms,” which includes sinonasal undifferentiated carcinoma (SNUC), small cell undifferentiated neuroendocrine carcinoma, poorly differentiated squamous cell carcinoma, rhabdomyosarcoma, malignant melanoma, olfactory neuroblastoma, NUT

midline carcinoma, and hematolymphoid malignancies [1–4]. Despite the varying histogenesis of these undifferentiated neoplasms, many times they share histological features that makes differentiating them quite challenging. The importance of accurately diagnosing these malignancies rests in the rapidly-expanding treatment options that may be tumor-specific, thus, drastically impacting a patient's survival. Immunohistochemical techniques, molecular studies, and chromosome analyses are often necessary to differentiate these neoplasms.

SNUC is a highly aggressive neoplasm of the nasal cavity and paranasal cavity without squamous or glandular differentiation that typically presents at an advanced stage and has a poor prognosis. Our study focuses on differentiating sinonasal undifferentiated carcinoma (SNUC) from other poorly differentiated neoplasms by further defining the immunohistochemical profile of SNUC, as well as investigating its possible association with human papillomavirus and expression of HPV DNA markers. It was hypothesized that these markers could be mechanistic in the disease process or of possible therapeutic importance.

Materials and Methods

All diagnoses of SNUC between 2000 and 2007 from the Departments of Pathology, University of Louisville Hospital and Norton Hospital, Louisville, Kentucky were reviewed and confirmed by two independent pathologists. Poorly differentiated squamous cell carcinoma (PDSCC) was chosen as the study control, given its well-reported immunohistochemical profile and very low frequency of HPV detection. The inclusion criterion for a poorly differentiated squamous cell carcinoma case was its absence of keratinization.

Our definition of SNUC tumors relied on the combination of histologic features and immunohistochemical profile in order to rule out other poorly differentiated neoplasms. The histologic criteria included poorly differentiated tumors with polygonal, hyperchromatic, and pleomorphic cells with frequent atypical mitotic activity and necrosis. No evidence of keratinization or recognizable differentiation along other cell lineages was present. By immunohistochemistry, SNUC tumors were expected to stain positively for CK19, EMA, and neuron specific enolase (NSE). SNUC tumors were expected to be negative for cytokeratins 5/6 and 14, S-100, CD45, MSA and desmin, to rule out squamous cell carcinoma, melanoma, lymphoma, and sarcoma, respectively. Although variable focal reactivity can be identified for p63 in SNUC, it is unexpected to see diffuse staining in SNUC, and this is important in ruling out squamous cell carcinoma.

Paraffin-embedded tissue blocks from all identified cases were selected for analysis. Eight-micrometer sections of tissue were deparaffinized and treated for antigen retrieval and subjected to an immunohistochemical panel that included: CK7 (Dako, Carpinteria, CA), CK19 (Dako, Carpinteria, CA), EMA (Dako, Carpinteria, CA), NSE (Zymed, Carlsbad, CA), chromogranin (Zymed, Carlsbad, CA), p53 (Dako, Carpinteria, CA), CK5/6 (Dako, Carpinteria, CA), p63 (Cell Marque, Rocklin, CA), CK14 (Novacastra, Bannockburn, IL), S100 (Signet, Dedham, MA), HMB-45 (Dako, Carpinteria, CA), desmin (Dako, Carpinteria, CA), muscle specific actin (Dako, Carpinteria, CA), and CD45 (Dako, Carpinteria, CA). Immunohistochemical stains (IHCS) were evaluated based on the percentage of tumor cells staining: <5% = negative, 5–50% = focally positive, 51–85% = positive, >85% = diffusely positive.

Additionally, tests for p16 (p16INK4a kit; Dako, Carpinteria, CA), EBV and HPV types 6/11, 16/18, 31/33/51 (Zymo Research, Orange, CA) were performed with appropriately reacting positive and negative controls. Following deparaffinization, total cellular DNAs were extracted from formalin-fixed, paraffin-embedded sections. In situ hybridization (ISH) was used to detect HPV DNA types 6, 11, 16, 18, 31, 33, and 51, and confirmed by classical polymerase chain reaction (PCR). PCR was carried out for 40 cycles using platinum tag polymerase and a set of degenerate primers (HPV-MY09; HPV-MY11, HPV-GP5, and HPV-GP6) capable of binding DNAs of 23 mucosotrophic HPV types. Touchdown PCR, more sensitive for detection of HPV, was performed simultaneously and compared with the results obtained by the classical PCR method. For quality control, beta-globin was amplified separately using a set of specific primers to ensure the presence of sufficient DNA for each PCR reaction. Conventional and touchdown PCR methods with platinum taq polymerase were also used to test the samples for EBV. The products were run separately on 3% agarose gels and sequenced to verify the presence of HPV or EBV in the samples. These methods were previously described by Desai et al. [5].

Results

A total of five patients (one woman and four men) with SNUC, ranging in age from 26 to 75 years (mean 56.8 years) and five patients (five men) with PDSCC, ranging in age from 53 to 75 years (mean 62.2 years) were included. The histologic diagnosis of SNUC and PDSCC were confirmed in all cases by H&E evaluation histology and IHCS (Figs. 1, 2).

All SNUC cases demonstrated diffuse nuclear and cytoplasmic positivity with p16 (Fig. 3), whereas only two

Fig. 1 Sinonasal undifferentiated carcinoma

Fig. 2 Poorly differentiated non-keratinizing squamous cell carcinoma

Fig. 3 Sinonasal undifferentiated carcinoma displays strong diffuse nuclear and cytoplasmic positivity for p16

of five PDSCC controls were positive for p16. HPV DNA was not detected in any of the SNUC cases or PDSCC controls. Touchdown PCR for HPV DNA was confirmatory in all cases. All cases of PDSCC diffusely expressed p63, whereas p63 was expressed focally in only two cases of SNUC. EBV, S100, HMB45 and LCA were negative in all cases of SNUC and PDSCC (Table 1).

Discussion

First described in 1986 by Frierson et al. [6], sinonasal undifferentiated carcinoma (SNUC) is an uncommon and highly aggressive neoplasm of the nasal cavity and paranasal sinuses. It has a slight male predominance (2–3:1) and can affect a broad age range (third to ninth decades), although it most commonly occurs in the sixth decade [1]. Most patients present with symptoms including epistaxis, rhinorrhea, chronic sinusitis, or nasal obstruction. It may arise in the nasal cavity, maxillary antrum or ethmoid sinus alone or in combination [1]. Unfortunately, most SNUC tumors are already large and infiltrative with poorly defined margins at the time of diagnosis. Radiographically, SNUC tumors are seen as expansive soft tissue masses that erode and invade adjacent bone rather than remodeling it [7].

SNUC is of uncertain histogenesis, but likely arises from the schneiderian epithelium [2]. Its appearance is characterized by a hypercellular proliferation of undifferentiated blue cells that display various growth patterns including trabecular, sheet-like, ribbons, lobular, or organoid [6].

In situ mucosal carcinoma or ulceration of the overlying mucosa is common. The cells are polygonal, pleomorphic with medium to large hyperchromatic nuclei, occasionally prominent nucleoli, a high nuclear-to-cytoplasmic ratio, and may have distinct or poorly defined cell borders. The cells may display evidence of neuroendocrine differentiation but lack glandular and squamous differentiation, true neural rosettes, and a fibrillary background [7]. There are frequent mitotic figures, prominent necrosis and apoptosis, as well as lymphovascular invasion. The SNUC cases we examined displayed moderately sized cells predominantly forming lobules and sheets. The oval-shaped cells had medium to large hyperchromatic nuclei, inconspicuous to prominent nucleoli, varying amounts of eosinophilic cytoplasm and ill-defined cell membranes. A significant amount of mitotic activity, including atypical forms, was present in all cases. Prominent confluent necrosis as well as individual cell necrosis was also noted. No squamous differentiation or rosette formation was identified (Fig. 1).

The differential diagnosis of SNUC includes other round blue cell neoplasms, such as poorly differentiated squamous cell carcinoma, undifferentiated nasopharyngeal carcinoma, olfactory neuroblastoma, NUT midline carcinoma, small

Table 1 Staining profiles comparing poorly differentiated squamous cell carcinoma to sinonasal undifferentiated carcinoma

Age	Gender	HPV	EBV	CK7	CK19	EMA	NSE	Chromogranin	p53	CK 5/6	CK 14	p63	Desmin	MSA	LCA	HMB-45	S100	p16
Poorly differentiated squamous cell carcinoma																		
53	M	–	–	+	f	N/A	+	–	N/A	f	f	+	f	–	–	–	–	–
55	M	–	–	f	+	+	+	N/A	f	+	f	+	f	f	–	–	–	–
64	M	–	–	–	–	+	f	f	–	N/A	–	+	–	–	–	–	–	–
75	M	–	–	+	+	+	–	–	f	+	+	+	–	–	–	–	–	+
64	M	–	–	–	f	f	f	–	+	f	–	+	–	–	–	–	–	+
Sinonasal undifferentiated carcinoma																		
66	M	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	+
54	M	–	–	–	f	–	f	–	+	–	–	–	–	–	–	–	–	+
26	M	–	–	–	–	+	+	–	+	–	–	–	–	–	–	–	–	+
63	F	–	–	f	+	+	+	–	–	f	–	f	–	–	–	–	–	+
75	M	–	–	N/A	+	f	–	–	–	f	f	f	–	–	–	–	–	+

+ Positive, – negative, f focal

cell neuroendocrine carcinoma, melanoma, lymphoma, extramedullary plasmacytoma, invasive/ectopic pituitary adenoma, embryonal rhabdomyosarcoma, and Ewing sarcoma/peripheral neuroectodermal tumor [3, 4]. While light microscopy may reveal some differences, often immunohistochemical techniques are necessary to aid in the distinction of SNUC from other high-grade neoplasms. Franchi et al. studied the cytokeratin (CK) expression patterns of SNUC and found it to express cytokeratins of simple epithelia, including CK8 (100% of cases), CK7, and CK19 (50% of cases). In comparison, they found that poorly differentiated squamous cell carcinoma (PDSCC), non-keratinizing squamous cell carcinoma (NKSCC), and nasopharyngeal type undifferentiated carcinoma showed immunoreactivity to CK5/6, CK8, CK13, and CK19. PDSCC and NKSCC also expressed CK14 [8]. The NUT immunohistochemical stain has been recommended as a more definitive way to exclude this entity from the differential, however, it was unavailable at our institution [4].

SNUC is inconsistently immunoreactive for epithelial membrane antigen (EMA), NSE, and p53, as was demonstrated by our cases [7]. Positivity for synaptophysin, chromogranin, S100 protein, or Leu-7 is rarely documented. Vimentin, muscle markers (desmin, myoglobin, myf4, actins), hematolymphoid markers (leukocyte common antigen, B and T cell lineage), and melanocytic cell markers (HMB-45, melan A) and CD99 are uniformly negative [3], which held true in our study. A publication in the American Journal of Surgical Pathology in 2001 described the immunohistochemical profile of 25 SNUC cases. All tumors were negative for EBER-1 by ISH, but showed variable positivity for Ki-67, p53, CD99, EMA and NSE [9]. In comparison, squamous cell carcinoma will stain positively for CK14 and p63, while sinonasal neuroendocrine carcinoma (SNEC) stains positively for chromogranin and S100.

EBV has been investigated as a possible causative agent in both sinonasal papillomas and SNUC. Dunn et al. [10] performed PCR analysis of 25 sinonasal papillomas, 23 of which did not express EBV. SNUC is also typically negative for Epstein-Barr virus (EBV) [9]. Jeng et al. [11] found 36 SNUC tumors to be negative for EBV DNA by in situ hybridization, although Gallo et al. [12] detected EBV DNA in 5 of 13 SNUC samples analyzed. All SNUC cases in our study were negative for EBV DNA.

It has been published that low risk HPV DNA (types 6 and 11) is a major etiological factor in the development of benign papillomas of the larynx and sinonasal tract. It has been published that low risk HPV-6 and HPV-11 DNA are present in up to 100% of recurrent respiratory papillomatosis cases, and in their subsequent laryngeal cancers [13, 14]. Syrjänen reviewed 1,041 cases of sinonasal papillomas and reported that HPV-6 and HPV-11 were present in 33.3% [15].

Additionally, carcinomas of the nose and paranasal sinuses have been found to harbor high-risk HPV DNA (types 16 and 18). Syrjänen reviewed 22 studies that analyzed 322 cases of sinonasal squamous cell carcinoma, and found HPV DNA was detected in 70 cases (21.7%) [15]. HPV-16 was the most frequent type, followed by HPV-18. However, no cases of SNUC were included in the study. It was published in the *New England Journal of Medicine* in 2007 that tonsillar carcinomas to have a higher prevalence of HPV at 51%, compared to 22% of oral carcinomas and sinonasal carcinomas, with HPV-16 as the most frequently isolated genotype (Syrjänen NEJM) [16]. El-Mofty et al. investigated HPV positivity in 39 cases of sinonasal carcinoma and found HPV DNA expression in 50% of non-keratinizing squamous cell carcinoma (4 of 8 cases), 19% of keratinizing squamous cell carcinoma (4 of 21 cases), and 10% of SNUC (1 of 10 cases). In contrast to our study, they reported negative or weakly reactive p16 staining in SNUC [17]. Rassekh et al. [18] found a coexistence of EBV and HPV DNA in undifferentiated nasopharyngeal carcinoma (NPC) and nasopharyngeal SCC. Of the 17 total cases, EBV was identified in 15 and HPV in 9 cases. All HPV-positive cases were also EBV positive. The percentage of sinonasal and oropharyngeal cancers that express HPV DNA is quite variable, possibly due to the small number of cases, the different HPV DNA detection methods used, the limited number of DNA genotypes included in the detection method, or the different sensitivities of various PCR primer sets used.

Recently, studies have investigated the presence of HPV as a prognostic indicator for head and neck cancer. It has been reported that HPV-positive cancers of the head and neck, including the tonsil, oropharynx, and oral cavity have a better overall survival than HPV-negative cancers [16, 19–23].

HPV-16 and 18 cause neoplasia by infection of immature epithelial squamous cells. The papillomavirus displays oncoproteins E6 and E7, which are always expressed in HPV-induced cancers and are required for maintaining the malignant state. The E6 and E7 oncoproteins bind to p53 and pRb, respectively, to inactivate their tumor suppression properties, thus, impairing cell cycle arrest. E7 binding also causes up-regulation of p16, a biomarker of HPV16 infection [25, 26].

Cancers commonly contain alterations involving the p16 gene, a cyclin kinase inhibitor and tumor suppressor gene. The p16^{INK4A} protein prevents phosphorylation of the Rb tumor suppressor protein and negatively regulates the cell cycle [27]. Evidence suggests that overexpression of the p16^{INK4A} protein indicates infection with and genomic integration of high-risk HPV [28]. Despite overexpression of p16, HPV-infected cells continue to proliferate because

Rb, the target of p16 inhibitory activity, is inactivated by the E7 HPV oncoprotein [29]. Therefore, p16 has been used as a predictor of cervical high-grade squamous intraepithelial lesions and carcinoma due to HPV-induced cell cycle dysregulation [28].

While p16 may be a sensitive marker of cells with active expression of E7 oncoprotein, its expression must be interpreted cautiously because it is not specific for HPV infection. Benign mucosa and carcinomas unrelated to HPV infection may also stain strongly with p16. Expression of p16 was detected in all epithelial cell layers of normal respiratory epithelium, sinonasal papillomas and adjacent mucosa [30]. In contrast to our study which found diffuse positivity for p16 in SNUC alone, El-Mofty et al. 2005 found SNUC tumors to be negative or weakly reactive for p16. However, their results were obtained using a different monoclonal antibody (p16, 1:40, Novacastra Labs, Ltd, UK). Schwerer also detected expression of p16 in sinonasal SCC. Klussman et al. 2009, demonstrated that p16^{INK4A} is highly expressed in HPV-related oropharyngeal SCC. The study found HPV-positivity in 55% of 57 cases of oropharyngeal SCC, of which 97% of the HPV-positive tumors showed strong p16^{INK4A} expression. Strong p16^{INK4A} staining was defined as diffuse nuclear as well as cytoplasmic staining in greater than 60% of the tumor cells. Only cases with positive HPV-PCR and p16 overexpression were regarded as HPV-related oropharyngeal SCC, as previously suggested [22, 32]. Thus, p16^{INK4A} immunostaining is a reliable surrogate marker to HPV-PCR to identify HPV-related tumors [22, 24, 31, 32].

The treatment for SNUC includes intensive multimodality therapy, including surgical resection and adjuvant chemoradiation for cases that are locally advanced and non-metastatic [33]. Despite aggressive management, the prognosis remains dismal with a median survival of less than 18 months and a 20% 5-year survival [34].

Conclusion

In conclusion, we found an over expression of p16 in SNUC occurring in the absence of HPV DNA expression. This report raises the question of whether p16 has a role in the carcinogenic process of SNUC or is merely suggestive of residual epithelial p16 staining, which is normally present in the sinonasal tract. It is clear that the number of SNUC cases studied is far too few to confirm the absence of HPV DNA in SNUC. Due to the rarity of this neoplasm, the lack of causal association will need to be further explored with additional SNUC cases that arise in the future.

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References

1. Frierson HF Jr. Sinonasal undifferentiated carcinoma. In: Barnes L, Eveson JW, Reichart P, Sidransky D, editors. World Health Organization classification of tumors. Lyon: IARC Press; 2005. p. 19.
2. Pilch BZ. Tumors of the upper respiratory tract, part B: larynx and trachea. In: Fletcher CD, editor. Diagnostic histopathology of tumors. 3rd ed. Philadelphia, PA: Churchill Livingstone; 2007. p. 112–5.
3. Hunt JL, Barnes L. Immunohistology of head and neck neoplasms. In: Dabbs D, editor. Diagnostic immunohistochemistry. 2nd ed. Philadelphia, PA: Churchill Livingstone; 2006. p. 232–5.
4. Stelow EB, Bellizzi AM, Taneja K, et al. NUT rearrangement in undifferentiated carcinomas of the upper aerodigestive tract. *Am J Surg Pathol*. 2008;32(6):828–34.
5. Desai PC, Jaglal MV, Gopal P, et al. Human papillomavirus in metastatic squamous carcinoma from unknown primaries in the head and neck: a retrospective 7 year study. *Exp Mol Pathol*. 2009;87(2):94–8.
6. Frierson HF Jr, Mills SE, Fechner RE, et al. Sinonasal undifferentiated carcinoma: an aggressive neoplasm derived from Schneiderian epithelium and distinct from olfactory neuroblastoma. *Am J Surg Pathol*. 1986;10(11):771–9.
7. Smith SR, Som P, Fahmy A, et al. A clinicopathological study of sinonasal neuroendocrine carcinoma and sinonasal undifferentiated carcinoma. *Laryngoscope*. 2000;110(10 Pt 1):1617–22.
8. Franchi A, Moroni M, Massi D, et al. Sinonasal undifferentiated carcinoma, nasopharyngeal-type undifferentiated carcinoma, and keratinizing and nonkeratinizing squamous cell carcinoma express different cytokeratin patterns. *Am J Surg Pathol*. 2002;26(12):1597–604.
9. Cerilli LA, Holst VA, Brandwein MS, et al. Sinonasal undifferentiated carcinoma: immunohistochemical profile and lack of EBV association. *Am J Surg Pathol*. 2001;25(2):156–63.
10. Dunn ST, Clark GD, Cannon TC, et al. Survey of sinonasal inverted papilloma for Epstein-Barr virus. *Head Neck*. 1997;19(2):98–106.
11. Jeng YM, Sung MT, Fang CL, et al. Sinonasal undifferentiated carcinoma and nasopharyngeal-type carcinoma: two clinically, biologically, and histopathologically distinct entities. *Am J Surg Pathol*. 2002;26(3):371–6.
12. Gallo O, Di Lollo S, Graziani P, et al. Detection of Epstein-Barr virus genome in sinonasal undifferentiated carcinoma by use of in situ hybridization. *Otolaryngol Head Neck Surg*. 1995;112: 659–64.
13. Major T, Szarka K, Sziklai I, et al. The characteristics of human papillomavirus DNA in head and neck cancers and papillomas. *J Clin Pathol*. 2005;58(1):51–5.
14. Reidy PM, Dedo HH, Rabah R, et al. Integration of human papillomavirus type 11 in recurrent respiratory papilloma-associated cancer. *Laryngoscope*. 2004;114(11):1906–9.
15. Syrjänen KJ. HPV infections in benign and malignant sinonasal lesions. *J Clin Pathol*. 2003;56(3):174–81.
16. Syrjänen S. Human papillomaviruses in head and neck carcinomas. *N Engl J Med*. 2007;356(19):1993–5.
17. El-Mofty SK, Lu DW. Prevalence of high-risk human papillomavirus DNA in nonkeratinizing (cylindrical cell) carcinoma of the sinonasal tract: a distinct clinicopathologic and molecular disease entity. *Am J Surg Pathol*. 2005;29(10):1367–72.
18. Rassekh CH, Rady PL, Arany I, et al. Combined Epstein-Barr virus and human papillomavirus infection in nasopharyngeal carcinoma. *Laryngoscope*. 1998;108(3):362–7.
19. Ritchie JM, Smith EM, Summersgill KF, et al. Human papillomavirus infection as a prognostic factor in carcinomas of the oral cavity and oropharynx. *Int J Cancer*. 2003;104(3):336–44.
20. Badaracco G, Rizzo C, Mafera B, et al. Molecular analyses and prognostic relevance of HPV in head and neck tumours. *Oncol Rep*. 2007;17(4):931–9.
21. Fakhry C, Westra WH, Li S, et al. Improved survival of patients with human papillomavirus—positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst*. 2008;100:261–9.
22. Klussmann JP, Mooren JJ, Lehnen M, et al. Genetic signatures of HPV-related and unrelated oropharyngeal carcinoma and their prognostic implications. *Clin Cancer Res*. 2009;15(5):1779–86.
23. D'Souza G, Kreimer AR, Viscidi R, et al. Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med*. 2007;356(19):1944–56.
24. Reimers N, Kasper HU, Weissenborn SJ, et al. Combined analysis of HPV-DNA, p16 and EGFR expression to predict prognosis in oropharyngeal cancer. *Int J Cancer*. 2007;120(8):1731–8.
25. McKaig RG, Baric RS, Olshan AF. Human papillomavirus and head and neck cancer: epidemiology and molecular biology. *Head Neck*. 1998;20(3):250–65.
26. Ragin CC, Modugno F, Gollin SM. The epidemiology and risk factors of head and neck cancer: a focus on human papillomavirus. *J Dent Res*. 2007;86(2):104–14. Review.
27. Pietruszewska W, Rieske P, Murlewska A, et al. Expression of p16 and protein in the evaluation of dynamics of laryngeal cancer growth. *Otolaryngol Pol*. 2004;58(1):173–80.
28. Holladay EB, Logan S, Arnold J, et al. A comparison of the clinical utility of p16(INK4a) immunolocalization with the presence of human papillomavirus by hybrid capture 2 for the detection of cervical dysplasia/neoplasia. *Cancer*. 2006;108(6):451–61.
29. Serrano M. The tumor suppressor protein p16INK4a. *Exp Cell Res*. 1997;237(1):7–13.
30. Schwerer MJ, Sailer A, Kraft K, et al. Expression of retinoblastoma gene product in respiratory epithelium and sinonasal neoplasms: relationship with p16 and cyclin D1 expression. *Histol Histopathol*. 2003;18(1):143–51.
31. Klussmann JP, Gultekin E, Weissenborn SJ, et al. Expression of p16 protein identifies a distinct entity of tonsillar carcinomas associated with human papillomavirus. *Am J Pathol*. 2003;162: 747–53.
32. Smeets SJ, Hesselink AT, Speel EJ, et al. A novel algorithm for reliable detection of human papillomavirus in paraffin embedded head and neck cancer specimen. *Int J Cancer*. 2007;121(11): 2465–72.
33. Gorelick J, Ross D, Marentette L, et al. Sinonasal undifferentiated carcinoma: a case series and review of the literature. *Neurosurgery*. 2000;47(3):750–5.
34. Thompson L. Malignant neoplasms of the nasal cavity, paranasal sinuses, and nasopharynx. In: Thompson L, editor. Head and neck pathology. Philadelphia: Churchill Livingstone; 2006. p. 168–71.