

Loss of p16 expression is a risk factor for recurrence in sinonasal inverted papilloma*

Marta Menéndez del Castro^{1,#}, Virginia Naves Cabal^{2,#}, Blanca Vivanco³, Laura Suárez-Fernández², Fernando López¹, José Luis Llorente¹, Mario A. Hermsen², César Álvarez-Marcos¹

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¹ Department of Otolaryngology, Hospital Universitario Central de Asturias, Oviedo, Spain

² Department of Head and Neck Oncology, Instituto de Investigación Sanitaria del Principado de Asturias, Oviedo, Spain

³ Department of Pathology, Hospital Universitario Central de Asturias, Oviedo, Spain

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contributed equally

Abstract

Background: The purpose of this study was to evaluate p16, p53, EGFR, pEGFR protein expression and HPV infection as possible markers of tumor progression in a series of sinonasal inverted papilloma (SNIP) and sinonasal squamous cell carcinoma (SNSCC).

Methods: A series of 49 SNIP, 11 SNSCC associated with SNIP (SNIP-SNSCC) and 52 SNSCC not associated with SNIP were analyzed for p16, p53, EGFR, and phosphorylated EGFR (pEGFR) expression by immunohistochemistry. Human papillomavirus (HPV) infection status was evaluated by DNA-PCR. Results were correlated to clinical and follow-up data.

Results: Reduced or loss of p16 expression was observed in 18% SNIP, 64% SNIP-SNSCC and 87% of SNSCC. Reduced or loss p16 staining in SNIP correlated with shorter recurrent SNIP-free follow-up. In contrast, p16 expression was not predictive of recurrent SNSCC in cases with SNIP-SNSCC and SNSCC. P53, EGFR, and pEGFR expression did not differ between the tumor groups, nor were they related to recurrent SNIP-free follow-up or recurrent SNSCC. Oncogenic HPV types 16 and 18 were detected in 5% of SNIP and 18% of SNIP-SNSCC, but not in SNSCC. There was no correlation between HPV infection and >70% p16 immunostaining.

Conclusions: HPV infection appears to play a minor role in SNIP and SNSCC and p16 immunostaining does not appear a valid surrogate marker for HPV. However, reduced or loss p16 expression may have prognostic value as a risk marker for recurrent SNIP.

Key words: sinonasal inverted papilloma, sinonasal squamous cell carcinoma, recurrence, HPV, p16

Introduction

Sinonasal papillomas are defined as a benign epithelial tumor composed of well-differentiated columnar or ciliated respiratory epithelium with a variable degree of squamous differentiation. Three different histopathological types have been described according to the World Health Organization (WHO): exophytic papilloma, columnar cell papilloma or oncocytic papilloma and Schneiderian or inverted papilloma (SNIP)⁽¹⁾. SNIP is a relatively uncommon tumor, accounting for 0.5 to 7% of all neoplasms of the sinonasal tract, with an incidence between 0.2 to 1.5/100.000 per year⁽¹⁻³⁾. Its etiology is unknown but different factors have been suggested, including human papillomavirus (HPV) infection and occupational exposure to organic solvents

^(1,4).

SNIPs are histologically benign, however, they may eventually have an aggressive behavior due to three special characteristics: tendency to local invasion and aggressive growth, local recurrence and malignant transformation into sinonasal squamous cell carcinoma (SNSCC)⁽⁵⁻⁷⁾. With a lesser incidence also other tumors as adenocarcinoma, mucoepidermoid carcinoma, undifferentiated carcinoma, verrucous carcinoma or transitional cell carcinoma may arise from SNIP⁽⁸⁾. The incidence of SNSCC associated with SNIP (SNIP-SNSCC) ranges from 2 to 27% in the literature⁽⁹⁾. SNSCC can occur metachronous, arising in the first 6 months after the initial diagnosis, or more frequently synchronous⁽¹⁾.

Although genetic alterations in SNSCC are still little studied, it appears that many are similar to those found in described in head and neck squamous cell carcinoma (HNSCC). Particularly frequent are mutations in genes involved in cellular proliferation and cell cycle regulation, such as TP53, CDKN2A, EGFR, NF1 and HPV infection⁽¹⁰⁾. As they have also been detected in SNIP, these genetic alterations may be early events in tumorigenesis⁽¹¹⁻¹⁷⁾. HPV infection and EGFR mutation may also have clinical relevance as both have been associated with longer SNIP-free follow-up^(11,12,14).

Numerous reports have focused on the role of HPV in the development of SNIP and in their progression to SNSCC^(2,18). However, possibly due to different HPV detection methods, including those that use p16 protein overexpression as a surrogate marker, but perhaps also to geographic differences in HPV incidence, there is little agreement on the impact of HPV on SNIP⁽¹⁹⁾. Through interaction with oncoproteins E6, E7 and E5, transcriptionally active HPV is known to respectively affect the expression of p53, p16 and EGFR⁽²⁰⁻²²⁾. The purpose of this study was to evaluate p16, p53, EGFR, pEGFR protein expression and HPV infection as markers of tumor progression in a series of SNIP and SNSCC.

Materials and methods

Patients

Between January 1989 and December 2014, tissue samples were obtained from 49 cases with a confirmed diagnosis of SNIP, 11 with SNIP-SNSCC and 52 SNSCC without previous history of SNIP. All patients had not been treated previously and underwent surgery (in majority endoscopic) for curative purposes in our hospital. All clinical data are summarized in Table 1. This study was performed in accordance with and approved by the Institutional Ethics Committee of the Hospital Universitario Central de Asturias and by the Regional CEIC from Principado de Asturias (approval number: 07/16 for project CICPF16008HERM and 2020.048 for project FIS PI19/00191). Informed consent was obtained from all patients.

Immunohistochemistry

Protein expression was analyzed by immunohistochemistry on 3 µm sections of individual tissue blocks of SNIP and SNIP-SNSCC. For the samples of SNSCC we used previously created tissue microarray blocks containing three cores from different areas of the same tissue block for each tumor. Immunohistochemistry (IHC) was performed on an automatic staining workstation (Dako Autostainer Plus; DakoCytomation). The antibodies used for IHC were anti-p16 (clone E6H4, VentanaRoche mtm laboratories AG, Heidelberg, Germany), anti-p53 (DO-7, DAKO, Glostrup, Denmark), anti-EGFR (clone 2-18C9, DAKO, Glostrup, Denmark) and anti-pEGFR (clone D7A5, Cell Signaling Technology, Cambridge, UK). The immunostained tumor sections were evaluated

by two investigators (MM and BV) in a single review process, blinded to the patients' clinical data. In samples where there was discrepancy, it was solved afterwards by looking together using a multi-head microscope. Most of the literature on p16 in SNIP and SNSCC has focused on its role as surrogate marker of HPV and used >70% positivity as cut-off, however, in our study we also wanted to evaluate loss or reduction of p16 expression. Previous studies that have evaluated p16 expression levels all used a different grading, for example 6 grades with increments of 20%⁽²³⁾, 4 grades with increments of 25%⁽³⁾, or 4 grades of <5%, 5-20%, 20-50% and 50-100%^(20,24). We chose our grading to take into account the >70% cut-off (grade 3), to evaluate complete absence or 0% (grade 0), and two intermediate levels of 1-30% (grade 2) and 30-70% (grade 3). P53 immunostaining was evaluated as positive when >10% of the malignant cells showed nuclear staining. EGFR and pEGFR immunostaining was considered positive when moderate to strong membranous or cytoplasmic staining was observed in >10% of tumor cells; tumors with no or weak staining were regarded as negative.

HPV detection

PCR with MY11/GP6+ primers (site-directed L1 fragment of HPV) was performed to detect a broad spectrum of HPV genotypes. Briefly, the reaction contained 25 µl of reaction mixture containing 1x PCR buffer, 2 mmol/L MgCl₂, 50 µmol/L of each deoxynucleoside, 0.5 µmol/L of sense and antisense primers, 10 µl of DNA sample and 1 U Taq DNA polymerase (Promega Biotech Iberica S.L. Madrid, Spain). The PCR thermal profile was 35 cycles: denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 1 min, with an initial denaturation at 94°C for 5 min and a final extension at 72°C for 10 min. The amplified DNA fragments of approximately 200 bp were identified by electrophoresis in 1.5% agarose gel with ethidium bromide. All positive specimens for L1 fragment were tested by hybridization assays using type-specific probes for HPV⁽¹⁴⁾.

Statistical analysis

SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) was used to analyze correlations between clinical and immunohistochemical staining factors using univariate Pearson's and Fisher's exact chi-squared tests. Kaplan-Meier curves were undertaken for the evaluation of DFS by application of univariate logarithmic range test (Log-rank test). Multivariate Cox regression analysis was performed for factors possibly related to recurrent SNIP, recurrent SNSCC and survival. P-values < 0.05 were considered to indicate statistical significance.

Results

Clinical features and follow-up

All SNIP and SNSCC were completely resected, for stage T3 and T4 SNSCC often carried out 'piece meal' by endoscopic appro-

Table 1. Clinical and follow-up data.

	SNIP		p-value	SNSCC		p-value
	w/o rec	with rec		snip-snscc	snscc	
Sex, n (%)			0.574			0.039
Male	17/27 (63%)	12/22 (55%)		4/11 (36%)	37/52 (71%)	
Female	10/27 (37%)	10/22 (45%)		7/11 (64%)	15/52 (29%)	
Age, years			0.546			0.217
Mean (range)	64 (36-81)	61 (28-94)		60 (22-82)	66 (42-92)	
Symptoms, n (%)						
Nasal obstruction	26/27 (96%)	14/22 (64%)	0.007	9/11 (82%)	14/52 (45%)	0.166
Facial pain	2/27 (7%)	1/22 (5%)	1.000	7/11 (64%)	21/52 (68%)	1.000
Epistaxis	4/27 (15%)	1/22 (5%)	0.362	2/11 (18%)	9/52 (29%)	0.696
Rhinorrhoea	9/27 (33%)	6/22 (27%)	0.760	2/11 (18%)	9/52 (29%)	0.696
Other ^a	4/27 (15%)	2/22 (9%)	0.392	7/11 (64%)	13/52 (25%)	0.199
Primary location, n (%)			0.171			0.040
Maxillary sinus	9/27 (33%)	10/22 (46%)		7/11 (64%)	42/52 (81%)	
Frontal sinus	2/27 (7%)	5/22 (23%)		0/11 (0%)	0/52 (0%)	
Ethmoid sinus	5/27 (19%)	1/22 (4%)		2/11 (18%)	10/52 (19%)	
Nasal cavity	11/27 (41%)	6/22 (27%)		2/11 (18%)	0/52 (0%)	
Side, n (%)			0.245			0.235
Right	10/27 (37%)	6/22 (27%)		5/11 (46%)	15/52 (48%)	
Left	17/27 (63%)	14/22 (64%)		5/11 (46%)	16/52 (52%)	
Both	0 (0%)	2/22 (9%)		1/11 (9%)	0/52 (0%)	
Krouse T stage ^b, n (%)			0.025			NA
T1	9/27 (33%)	4/22 (18%)		NA	NA	
T2	15/27 (56%)	8/22 (36%)		NA	NA	
T3	3/27 (11%)	10/22 (46%)		NA	NA	
T stage n (%)			NA			0.847
T1	NA	NA		0/11 (0%)	0/52 (0%)	
T2	NA	NA		2/11 (18%)	5/52 (10%)	
T3	NA	NA		3/11 (27%)	16/52 (31%)	
T4a	NA	NA		4/11 (36%)	23/52 (44%)	
T4b	NA	NA		2/11 (18%)	8/52 (15%)	
N stage n (%)			NA			0.625
N0	NA	NA		6/11 (55%)	41/52 (79%)	
N1	NA	NA		5/11 (46%)	9/52 (17%)	
N2	NA	NA		0/11 (0%)	2/52 (4%)	
Differentiation			NA			0.991
well	NA	NA		4/11 (36%)	20/52 (39%)	
moderately	NA	NA		2/11 (18%)	9/52 (17%)	
poorly	NA	NA		5/11 (46%)	23/52 (44%)	
Complementary treatment, n (%)			NA			0.902
Radiotherapy	0 (0%)	0 (0%)		9/11 (81%)	38/52 (73%)	
Chemotherapy	0 (0%)	0 (0%)		0/11 (0%)	2/52 (4%)	
Recurrent SNSCC, n (%)	NA	NA	NA	10/11 (91%)	43/52 (83%)	0.676
Disease-free survival, months			NA			0.920
Mean (range)	NA	76 (11-318)		30 (2-159)	21 (1-216)	

	SNIP		p-value	SNSCC		p-value
	w/o rec	with rec		snip-snscc	snscc	
Overall survival, months			NA			0.562
Mean (range)	NA	NA		45 (3-159)	30 (1-216)	

w/o rec: without recurrent SNIP; with rec: with recurrent SNIP; NA: not applicable; ^a Other symptoms include exophthalmia, hyposmia, facial swallowing, oroantral fistulae, cervical mass; ^b Krouse T stage is used in SNIP only.

aches. Apparent clean tumor margins were observed by the surgeon and intraoperative biopsies from suspicious or doubtful areas were routinely sent to the pathologist to guarantee a complete resection. However, this does not guarantee pathologically confirmed free microscopic margins.

Of 49 SNIP patients, 20 were female (41%) and 29 male (59%). Mean age was 63 years (range 28-85). According to the Krouse classification, there were 13 T1 (27%), 23 T2 (46%) and 13 T3 (27%). Nineteen originated in the maxillary sinus (39%), 7 frontal sinus (14%), 6 ethmoid (12%) and 17 nasal cavity (35%). None of the 49 patients with SNIP received complementary radio- or chemotherapy after surgery. Twenty-two (45%) patients with SNIP developed a recurrent SNIP during follow-up, with a mean disease-free time of 76 months (range 11-318).

All 11 SNIP-SNSCC were of the keratinizing type, 7 women (64%) and 4 men (36%). Four had metachronous and 7 had synchronous SNIP. Mean age was 60 years (range 22-82). Seven tumors were located in the maxillary sinus (64%), 2 ethmoid (18%) and 2 nasal cavity (18%). Two cases were T2 (18%), 3 T3 (27%), 4 T4a (36%) and 2 T4b (18%). At the time of diagnosis, 5 (46%) patients harbored lymph node metastasis. All patients underwent radical surgery, while 9 (81%) received postoperatively radiotherapy; for two stage T2 patients surgery alone was considered sufficient. Mean follow-up time was 45 months (range 3-159). During follow-up, 10 (91%) patients developed recurrent SNSCC.

All 52 SNSCC were of the keratinizing type, 15 female (29%) and 37 male (71%). Mean age was 66 years (range 42-92). Forty-two (81%) tumors were localized in maxillary sinus and 10 (19%) ethmoid sinus. Five (10%) cases were tumor stage T2, 16 T3 (31%), 23 T4a (44%) and 8 T4b (15%). At the time of diagnosis, 11 (21%) patients harbored lymph node metastasis. All patients underwent radical surgery, while 38 (73%) received postoperatively radiotherapy and 2 (4%) chemotherapy; 12 patients did not receive adjuvant therapy due to early and extensive recurrence, high age and bad physical condition or refusal to receive further treatment. Mean follow-up time was 30 months (range 1-216). During follow-up, 43 (83%) developed recurrent SNSCC. A detailed description of all clinical and follow-up data is given in Table 1.

Immunohistochemical analysis

Nuclear and cytoplasmic p16 expression in >70% of tumor cells

was observed in 37% (10/27) of SNIP and in 32% (7/22) of SNIP that later developed a recurrent SNIP. In SNIP-SNSCC and SNSCC this was significantly less: 18% (2/11) and 11% (6/52) (Fisher's χ^2 p=0.001 and p=0.000), respectively (Table 2). Conversely, reduced or loss of p16 expression occurred in 7% (2/27) SNIP and 32% (7/22) SNIP that later developed a recurrent SNIP (Fisher's χ^2 p=0.006). In SNIP-SNSCC and SNSCC, this was 64% (7/11) and 87% (45/52), respectively (Fisher's Chi2 p=0.089) (Table 2). Comparing synchronous and metachronous SNIP-SNSCC, reduced or loss of p16 expression appeared more frequent in the former (6/7 versus 1/4 cases, Fisher's χ^2 p=0.088), although due to the low number of cases, this observation should be interpreted with caution.

There was no difference in p16 expression levels among the different anatomical localizations of the SNIP nor of the SNSCC. Representative images of p16 staining grades 0 to 3 both in SNIP as in SNSCC are given in Figure 1. Reduced or loss of p16 expression was observed more frequently in higher Krouse stage SNIPs, with p16 grades 0 and 1 in 0% (0/13) of Krouse stage T1 versus 13% (3/23) of stage T2 and 46% (6/13) of stage T3 SNIPs (Pearson's χ^2 p=0.052).

P53 expression was found in 56-69% of SNIP, SNIP-SCC and SNSCC (Table 2). EGFR immunohistochemistry failed for 2 SNIP and 5 SNIP-SNSCC cases; positivity was more frequent in SNIP (77-96%), than in SNIP-SNSCC (67%) and SNSCC (40%). Expression of pEGFR followed a similar pattern (Table 2). P53, EGFR and pEGFR expression (representative images of given in Figure 2) did not correlate to any of the clinical parameters or to p16 expression. HPV status could not be analyzed in 13 SNIP and 6 SNSCC cases. High-risk HPV types 16 and 18 were found in 5% (2/36) SNIP, in 18% (2/11) of SNIP-SNSCC and in 0% (0/46) of SNSCC. Statistical comparisons were performed (Table 2) but the low number of positive cases preclude strong conclusions. Low-risk HPV types 42, 56 and 61 were each found in one case of SNIP-SNSCC (Table 2). Again, due to the low number of HPV-positive cases, we did not analyze correlations with clinical or follow-up data. The two cases SNIP and the two cases SNIP-SNSCC with HPV16/18 infection showed a p16 immunostaining of less than 70% and were p53 positive, EGFR positive and pEGFR negative.

Correlation with follow-up data

Krouse stage 3 SNIPs showed a higher risk of developing a

Table 2. Immunohistochemical staining results of p16, p53, EGFR and pEGFR and HPV infection according to type of tumor sample.

	SNIP		p-value	SNSCC		p-value
	w/o rec	with rec		snip-snscc	snscc	
p16			0.060*			0.089*
grade 0: 0%	0/27 (0%)	4/22 (18%)		6/11 (55%)	45/52 (87%)	
grade 1: 1-30%	2/27 (7%)	3/22 (14%)		1/11 (9%)	0/52 (0%)	
grade 2: 31-70%	15/27 (56%)	8/22 (36%)		2/11 (18%)	1/52 (2%)	
grade 3: >70%	10/27 (37%)	7/22 (32%)		2/11 (18%)	6/52 (11%)	
p53 positive	15/27 (56%)	13/22 (59%)	1.000	7/11 (64%)	36/52 (69%)	0.732
EGFR positive	26/27 (96%)	17/20 (77%)	0.298	4/6 (67%)	21/52 (40%)	0.387
pEGFR positive	16/27 (59%)	12/22 (55%)	0.779	5/11 (45%)	19/52 (36%)	0.735
HPV						
types 16/18	2/20 (10%)	0/16 (0%)	0.492	2/11 (18%)	0/46 (0%)	0.034
types 42/56/61	0/20 (0%)	0/16 (0%)	NA	3/11 (27%)	0/46 (0%)	0.006

w/o rec: without recurrent SNIP; with rec: with recurrent SNIP; *comparison of p16 grades 0-1 versus 2-3.

Table 3. Univariate and multivariate Cox regression recurrent SNIP-free follow-up analysis of Krouse classification and p16 expression.

	Univariate			Multivariate	
	Recurrent SNIP	Log rank	Significance	HR (95% CI)	Significance
Krouse T stage					
1	2/13 (15%)				
2	5/22 (23%)	5.612	p=0.060	0.57 (0.23 - 1.44)	p=0.236
3	6/13 (46%)				
p16 expression					
0-10%	4/4 (100%)				
10-30%	2/5 (40%)				
30-70%	4/22 (18%)	18.470	p=0.000	2.17 (1.20 - 3.91)	p=0.010
70-100%	3/17 (18%)				

HR: Hazard ratio; CI : Confidence interval.

recurrent SNIP, with a 5-year DFS of 58%, whereas in Krouse stages 1 and 2 this was 84% and 91%, respectively (Log rank 5.612, p=0.060). None of the other clinical data were related to DFS. Immunohistochemical staining of p16 expression significantly (Log rank 18.470, p=0.000) correlated with increased risk of SNIP to develop a recurrent SNIP. Particularly, the lower p16 staining grades 0 and 1 showed a 5-year DFS of 25% and 53% versus 91% and 87% for the higher p16 staining grades 2 and 3, respectively (Figure 3). Multivariate Cox regression analysis was performed with p16 expression and Krouse stage, the only two parameters with significant or nearly significant differences in univariate analysis (Table 3) and showed that reduced and lost p16 expression in SNIP was a prognostic factor independent from Krouse stage for risk of developing recurrent SNIP (Hazard ratio 2.17, p=0.010). Expression of p53, EGFR and pEGFR was not related to DFS of SNIP. Analyzing SNIP-SNSCC and SNSCC, overall

and disease-free survival were comparable between the two subgroups. Expression of p16, p53, EGFR and pEGFR showed no correlation with overall and disease-free survival in SNIP-SNSCC or SNSCC.

Discussion

A wide variety of factors in SNIP have been related to recurrent SNIP and progression to SNSCC, including smoking, bone invasion, absence of inflammatory polyps, hyperkeratosis, presence of squamous epithelial hyperplasia and increased mitotic index^(1,4,7). Krouse and other classification systems have been claimed to have prognostic value for the risk of recurrent SNIP⁽²⁵⁻²⁷⁾ while recurrent SNIP itself may indicate an increased risk of developing malignant tumors⁽²⁸⁾. In our series, Krouse stage III SNIP indeed developed more frequent recurrent SNIP than stages I and II (Figure 3), similar to the findings of a large meta-study of

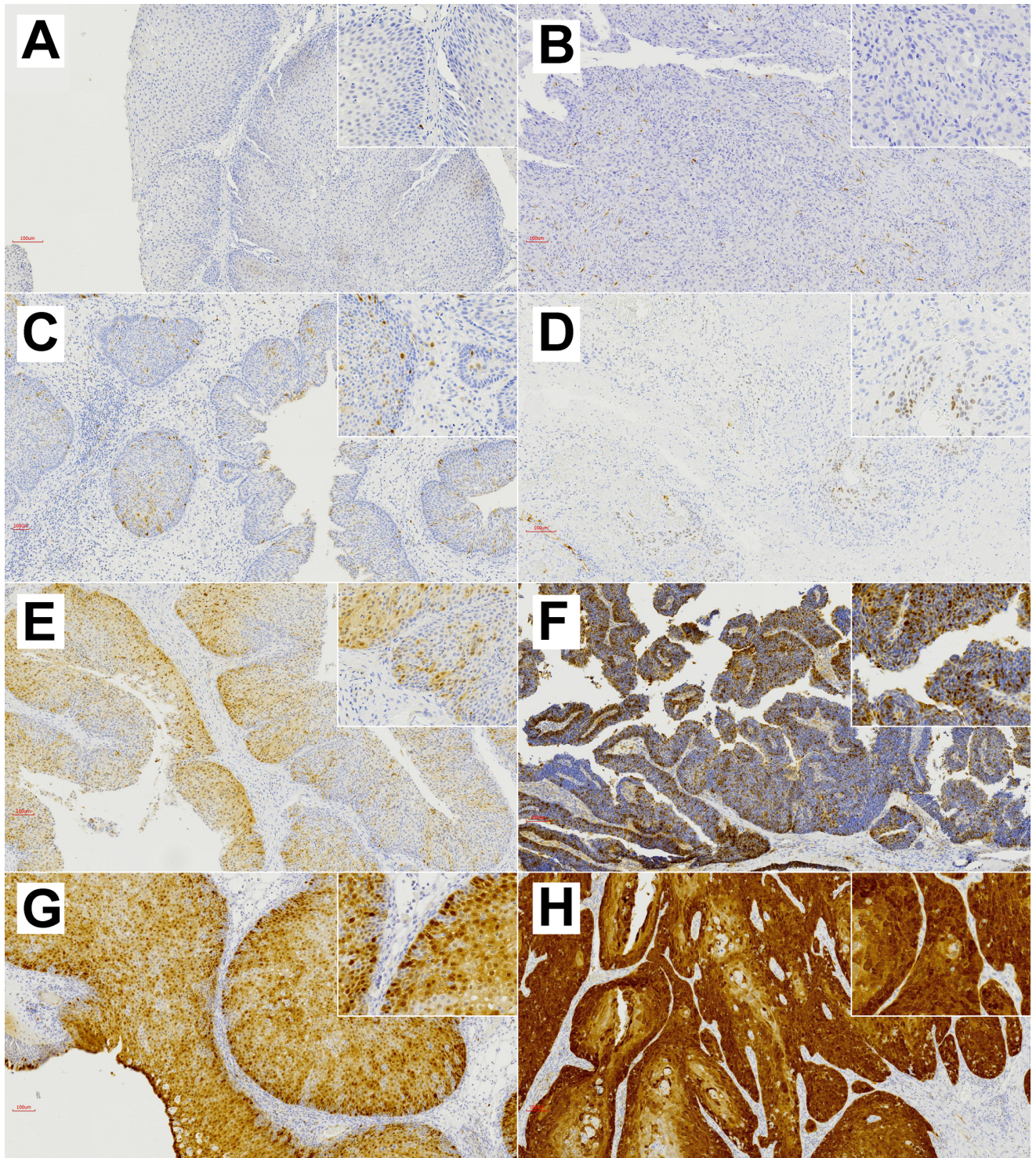


Figure 1. Photomicrographs of immunohistochemical expression of p16 in SNIP (A, C, E and G) and SNSCC (B, D F and H). Cytoplasmatic and nuclear staining of p16 was scored as grade 0 when negative (A, B), grade 1 for 1-30% (C, D), grade 2 for 30-70% (E, F) and grade 3 for >70% (G, H). Original magnification 10x and insert 20x.

1787 SNIPs⁽²⁷⁾.

In addition to clinical parameters, the prognostic potential of several immunohistochemical stainings such as EGFR, TGF α , desmoglein, SCCA, Ki67, E-cadherin and Beta-catenin^(7,29), as well as genetic mutations affecting TP53, CDKN2A and EGFR

have been studied⁽¹¹⁻¹⁷⁾. Many studies have been devoted to HPV and its effects on cell cycle, MAPK and PI3K signalling pathways. However, its role in SNIP and SNSCC is still a matter of debate, due to greatly varying reported frequencies and common findings of non-oncogenic low-risk HPV subtypes^(3-5,18-20,22,30). It

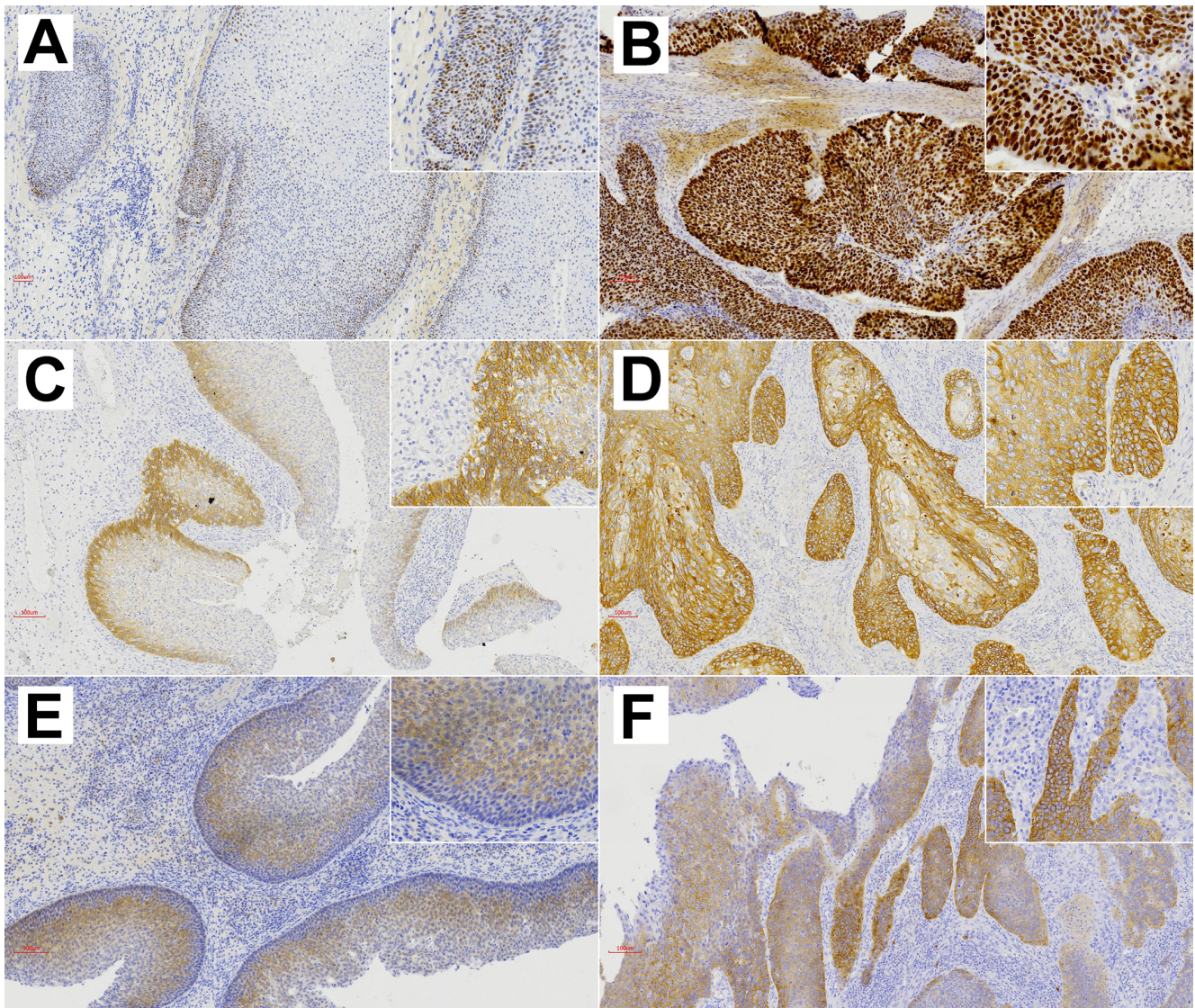


Figure 2. Photomicrographs of immunohistochemical expression of p53, EGFR and pEGFR in SNIP (A, C, and E) and SNSCC (B, D and F). Cases were evaluated as positive when >10% of the malignant cells showed nuclear staining for p53 (A, B) and moderate to strong membranous or cytoplasmic staining for EGFR (C, D) and pEGFR (E, F). Original magnification 10x and insert 20x.

may be argued that not the presence of HPV-DNA as such, but rather the transcriptional activity of HPV is biologically relevant in cancer. Unfortunately, few studies on SNIP and SNSCC have analyzed transcriptionally active HPV. Using HPV-RNA-ISH, transcriptionally active HPV has been detected in 18-29% of SNIP-SNSCC^(19,31-32). Notably, Rooper et al. found 0% of 52 SNIP to express HPV-RNA⁽¹⁹⁾. These studies may indicate that HPV does not play a role in malignant transformation of SNIP to SNSCC but may have some clinical relevance in SNSCC.

As the HPV E7 oncoprotein causes up-regulation of the cell cycle regulator p16, this protein has been studied in SNIP and SNSCC as a surrogate marker of transcriptionally active HPV infection, taking example from studies on oropharyngeal SCC^(33,34). Indeed, a positive correlation between transcriptionally active HPV and p16 positivity in >70% of tumor cells has been reported in

SNSCC^(19,31). However, there was no correlation between p16 expression and the detection of HPV-DNA^(4,20,23,35). Aside from its supposed relation to HPV, aberrant p16 expression may still be an important event in SNIP and SNSCC. In HPV-negative HNSCC it has been shown that p16 plays an important role in the upregulation of cell cycle signalling and CDKN2A (the encoding gene for p16) is the second most frequently mutated gene in HNSCC after TP53⁽¹⁰⁾. Two recent sequencing studies on SNIP-SNSCC have yielded similar results, with 33-42% of cases carrying inactivating CDKN2A mutations^(16,17). Aside from gene mutations, also frequent gene copy number losses have been found¹⁶. Discrepant results were obtained regarding SNIP, where Uchi et al. also found CDKN2A mutations in 2/3 cases, but Brown et al did not find any in 11 cases^(16,17), so more studies are needed.

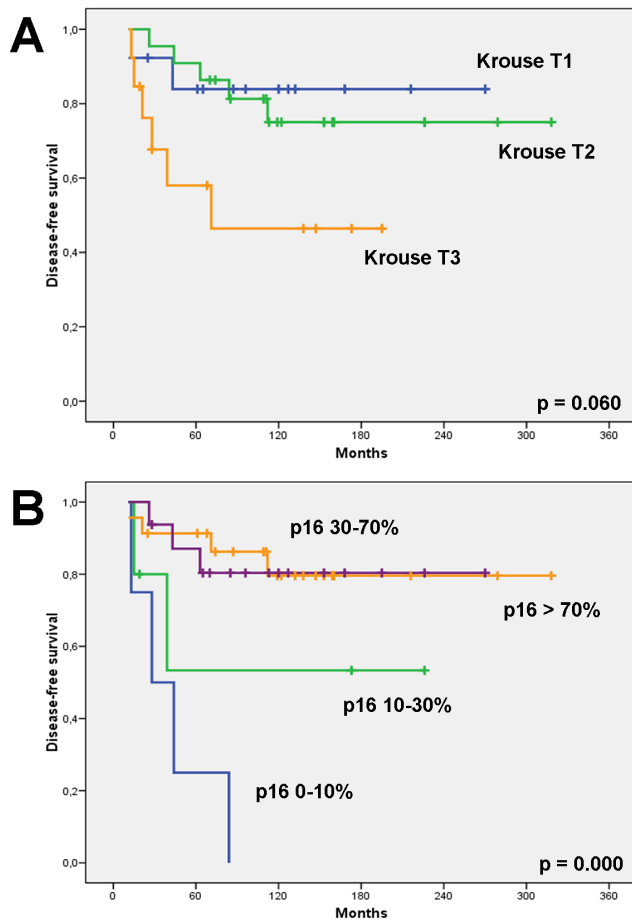


Figure 3. Recurrent SNIP-free follow-up of 49 SNIP according to Krouse T stage (A) and according to p16 scores (B).

Inactivating mutation and copy number loss of CDKN2A lead to reduced or complete absence of p16 expression, which can be analyzed by immunohistochemistry. Our results indicated 18% (9/49) SNIP to have lost (grade 0) or reduced (grade 1) p16 expression, while in SNIP-SNSCC and SNSCC this was 64% and 87%, respectively, suggesting that this genetic alteration is increasingly involved in the progression from SNIP to SNSCC. This is in agreement with Lin et al., who reported a significantly more frequent loss of p16 expression in 133 SNIP-SNSCC (84%) compared to 21 benign SNIP (36%)⁽²⁴⁾. Additionally, our study revealed reduced and loss of p16 expression in SNIP to be related to the risk of developing recurrent SNIP, and this prognostic value was independent from Krouse T stage (Figure 3 and Table 3). Studying 53 SNIPs, Zydron et al. did not find a significant correlation between the level of p16 expression and recurrent SNIP, however, comparison is difficult as the cut-off percentages for scoring were not detailed in their study⁽³⁶⁾. In addition, it is possible that data obtained from immunohistochemical stainings suffer from sampling bias. The series of SNIP in our study concerned whole paraffin blocks and the SNIP-SNSCC and SNSCC were represented each by three 1 mm cores in tissue

microarray blocks; it may be that the studied tissue areas did not fully represent the tumors. Another difference with previously published studies is the fact that both SNIP-SNSCC and SNSCC in our series carried a very high rate of recurrent SNSCC. This may reflect the distribution of tumor-stage: the proportion of T4a/T4b tumors is well over 50% of all cases. This in turn may be since our hospital is a center of referral for sinonasal and skull base tumors, therefore receiving a relatively high number of advanced stage patients.

This study has several limitations. First, our results related to recurrence outcomes are limited by the fact that tumor resection margins have not been evaluated routinely by the pathologist. Second, we have no information on the mutation and copy number status of CDKN2A as possible cause of the observed reduced or loss of p16 expression, and the preliminary data in this study will need to be validated by analysis of p16 expression on the mRNA level. A third limitation is the lack of SNIP cases that during follow-up developed SNSCC, which might shed further light on the importance of CDKN2A alterations and p16 expression in the process of malignant transformation. These aspects need to be investigated in future studies.

Conclusion

Altered p16 expression is a genetic event in SNIP that is relevant to be studied independent from its possible association with HPV infection. Our data are an indication that reduced or loss of p16 expression may be a risk factor for recurrent SNIP.

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Informed consent

Informed consent was obtained from all individual participants included in the study.

Authorship contribution

MM, VNC, JLL, CAM and MAH contributed to the study conception and design. MM, FL, CAM and JLL were responsible for tissue sample and clinical data collection. Material preparation, data collection and analysis were performed by MM, VNC, BV, LSF. The first draft of the manuscript was written by MM, VNC and MAH and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of interest

All authors declare they have nothing to disclose.

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Mario A. Hermsen

Grupo Oncología de Cabeza y Cuello

Instituto de Investigación Sanitaria

del Principado de Asturias (ISPA)

Instituto Universitario de Oncología

del Principado de Asturias (IUOPA)

Centro de Investigación Biomédica

en Red (CIBER-ONC)

Edif. FINBA, N-1 F49

C/ Avenida de Roma s/n

33011 Oviedo

Spain

Tel: +34-985107937

Fax: +34-985108015

E-mail: mhermsen@hca.es

ORCID nº 0000-0002-5959-6289