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p53 Over-expression and its correlation with PCNA index in nasal polyps*

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SUMMARY

Our knowledge about the etiopathogenesis of nasal polyps (Nps) is still limited. In this study, in order to define the biological features of these neoformations, we investigated with immunohistochemistry the p53 over-expression and the proliferating cell nuclear antigen (PCNA) in 32 cases of Nps and in normal mucosa of 11 control cases.
The evaluation of PCNA showed a wide range of indices (0.5-18.2%) with a mean value (6.8%) significantly higher than in normal mucosa (2.9%). Over-expression of the p53 oncoprotein, observed in 50% of Nps, was statistically related to a high PCNA-index (>6.8%).
Our results suggest that Nps can behave, in a high percentage of cases, like tumours.

Key words: epithelial cell proliferation, immunohistochemistry nasal polyps p53 mutation, proliferating cell nuclear antigen

INTRODUCTION

Nasal polyps (NPs) are peduncolated opalescent neoformations that arise from the mucosa of the nasal cavity. Their etiology and pathogenesis have still not been defined.

Nasal polyposis is frequently found in association with conditions such as cystic fibrosis, aspirin intolerance, asthma, rhinitis, thus suggesting a multifactorial etiopathogenesis (Schwachman et al.,1962; Samter and Beers 1968; Caplin et al.,1971; Small et al.,1981).

Histologically NPs consist of an edematous myxoid stroma infiltrated by inflammatory cells, for the most part eosinophils, overlaid by respiratory epithelium which frequently shows hyperplasia and squamous metaplasia.

According to Tos et al. (1977, 1990), these epithelial modifications are the first stage which leads to formation of Nps.

Coste et al. (1996 a,b) have suggested the important role of respiratory epithelium in nasal polyposis pathogenesis after demonstration that its proliferative activity is significantly higher than in normal mucosa.

Since the increased cell proliferation is frequently related to high aggressiveness, we tried to define the biological features of these neoformations. By using immunohistochemical methods in a large sample of Nps we evaluated the possible presence of p53 over-expression, a common denominator of different neoproliferative processes, and its correlation with the proliferating cell nuclear antigen (PCNA), a protein expressed in cycling cells.

MATERIAL AND METHODS

The study involved Nps surgically obtained from 32 patients (24 males and 8 females; mean age 48.2 years). The control group consists of inferior turbinate normal mucosa specimens excised from 11 patients (8 males and 3 females; mean age 46.8 years) with different nasal pathologies (chronic sinusitis, allergic rhinitis) or obtained during nasal reconstructive surgery. All subjects of this study were nonsmokers within the past 3 years.

The clinical diagnosis of Nps was established according to medical history, endoscopic findings and computed tomographic results.

Surgical samples were fixed in 10% buffered formalin and embedded in paraffin, according to routine histological procedures. Three serial 5μm thick sections were prepared for each case. One of these was stained with hematoxylin/eosin for histological examination and two were submitted to immunohistochemical analysis in order to determine the presence of the oncoprotein p53 and of PCNA, respectively.

Immunohistochemistry

p53: After blocking the formation of endogenous peroxidase the sections were incubated first in citrate buffer, then with the monoclonal antibody pAb 1801 (Oncogene Science, Inc., Manhasset, NY), directed against an epitope resistant to denaturation between the amino acids 32 and 79 of the protein, dilution 1:100, for one hour at room temperature. The sections were

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then incubated with the secondary antibody followed by the avidin-biotin-peroxidase complex. Diaminobenzidine was used as a substrate to visualize the enzymatic reaction. The preparations were finally contrasted with Mayer's hematoxylin.

The p53 evaluation was performed in epithelial cells by two independent observers. To minimize the subjectivity of interpretation, the prevalent staining intensity of cell nuclei (intensity score: 0= none; 1= weak; 2= moderate; 3= strong) was assigned to any case, as suggested by Allred et al., (1993), and the concordance of results was evaluated. Prevalent strong staining was interpreted as "over-expression" of the p53 protein.

PCNA: Sections were air-dried overnight at room temperature and immunostained with the monoclonal antibody PC 10 at a dilution of 1:200, using an immunoperoxidase method (ABC complex) with light hematoxylin counterstaining. All immunostained sections were examined using a 25× objective. The PCNA labeling index (PCNA-LI) was determined in respiratory epithelium from NPs and normal mucosa and was defined as the number of cells with strong unequivocal nuclear staining, corresponding to cells in S phase, divided by the total number of epithelial cells counted, expressed as percentage. The mean value was evaluated on the basis of the results obtained. Values above or equal to the mean were considered high PCNA-LI, those below the mean were considered low PCNA-LI.

Statistical analysis

Statistical analysis was carried out using the Chi-square test. The selected level of significance was $p<0.05$.

RESULTS

The results obtained from Nps by immunohistochemical analyses are presented in Table 1. Overall 24 cases (75%) showed positivity for p53 mutation in all layers of lining respiratory epithelium, with variations of immunoreactivity from 1 (weak) to 3 (strong). Concordance of interpretation was found between the two independent investigators in 91% of the cases. Discordant cases were discussed until consensus was reached.

Over-expression, as represented by diffuse distribution of strongly immunostained cells was present in 16 Nps, that is in 66% of the positive cases (16/24) and in 50% of all cases (16/32).

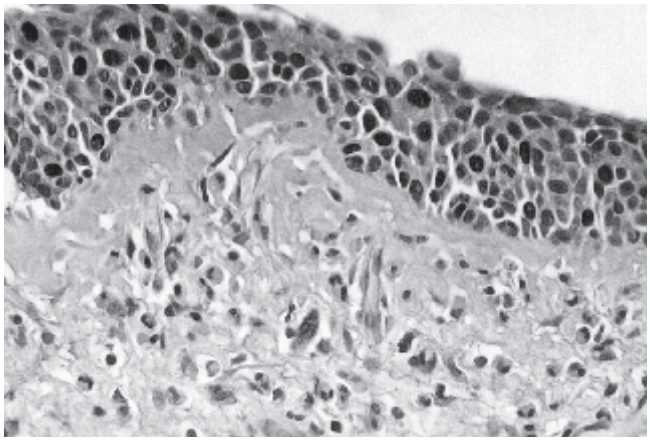

Figure 1. Over-expression of p53 oncoprotein in nasal polyps (× 250)

Table 1. Immunohistochemical results in nasal polyps

CASE	P53 STAIN INTENSITY				PCNA L.I.
No.	Absent (0)	Weak (1)	Moderate (2)	Strong (3)*	%
1				+	6.9 (H)
2			+		5.6 (L)
3	+				0.5 (L)
4				+	8.5 (H)
5				+	18.2 (H)
6	+				1.9 (L)
7		+			5.6 (L)
8				+	14.0 (H)
9	+				5.0 (L)
10	+				0.5 (L)
11				+	7.6 (H)
12				+	13.4 (H)
13				+	11.5 (H)
14		+			4.5 (L)
15				+	7.6 (H)
16				+	11.4 (H)
17	+				2.5 (L)
18			+		0.6 (L)
19				+	10.6 (H)
20				+	12.6 (H)
21				+	15.1 (H)
22				+	8.5 (H)
23			+		4.3 (L)
24		+			0.8 (L)
25		+			1.8 (L)
26	+				1.5 (L)
27	+				3.5 (L)
28	+				6.1 (L)
29		+			6.7 (L)
30				+	4.2 (L)
31				+	7.3 (H)
32				+	10.4 (H)

* = "Over-expression" of p53 oncoprotein
H = High proliferative activity (PCNA index ≥ 6.8%)
L = Low proliferative activity (PCNA index < 6.8%)

Table 2. Proliferative activity in nasal polyps and normal mucosa

	PCNA (%)	
	RANGE	MEAN VALUE
polyps	0.5 – 18.2	6.8
normal mucosa	0 – 8.2	2.9
		$p<0.05$

An example of strong intensity of p53 staining in polyp epithelium is given in Figure 1.

Immunoreactivity for p53, with an intensity score of 2 (moderate), was seen only in one control case (9%).

The evaluation of PCNA showed a wide range of indices, from 0.5 to 18.2%, with a mean value corresponding to 6.8%. Using

the mean as cut-off, 15 Nps (47%) were considered as highly proliferative (PCNA-LI 6.8%) and 17 (53%) had low proliferative activity (PCNA-LI< 6.8%).

Table 1 shows a significant correlation between p53 over-expression and a high proliferative activity. In fact, all the 15 cases with PCNA-LI 6.8% are characterized by a strong staining of the oncoprotein. Only one nasal polyp with a p53 mutation (case No.30) had a proliferative activity lower than the mean value (PCNA-LI=4.2%).

In the controls, the PCNA indices were significantly lower than those of Nps (range: 0-8.2%; mean value: 2.9%; $p<0.05$) (Table 2).

DISCUSSION

In order to understand the still unclear mechanism leading to growth of nasal polyps (NPs), we have investigated in this study some biological features usually involved in human malignancy. We looked in particular to the proliferating cell nuclear antigen (PCNA) and the p53 oncoprotein on the lining epithelium, which frequently in these neoformations shows morphological changes, such as hyperplasia and metaplasia.

Krajina in 1963 has already suggested that epithelial hyperplasia, combined with edema of the nasal mucosa, leads to creation of Nps.

Tos et al., (1977, 1990) suggested a pathogenetic theory, of which the first stage has been epithelial degeneration in areas where Nps are formed.

Recently, Coste et al., (1996 a,b) have suggested that epithelial cell proliferation, which is constantly higher than in normal mucosa, could play an important role in the pathogenesis of Nps.

We have also observed a significative increase of the PCNA labeling index in Nps compared with normal nasal mucosa, although our values are remarkably lower than those reported by these authors. This discrepancy is probably due to different criteria of PCNA evaluation.

In our study only intensely immunostained nuclei are considered as positive, that is cells in the S phase of DNA replication. In fact, PCNA expression, corresponding to the intensity of staining, is highly dependent on the cell cycle and it varies in the different phases. At the end of the G1 phase it begins to increase, reaching a maximum during the S phase and then decreases in the subsequent G2 stage and during mitosis. The most darkly stained cells, therefore, correspond to cells in the proliferative phase (Kurki et al.,1986; Garcia et al.,1989; Yu et al.,1991).

Other interesting results are obtained in the present study from the p53 evaluation.

The p53 gene is a tumour suppressor gene located on the short arm of chromosome 17, which has an important role in regulating cell cycle dynamics. The corresponding p53 protein, in fact, is able to inhibit DNA synthesis and cellular entry into S phase. It has a short half-life (20-30min) and is therefore expressed at low levels in normal cells (Levine et al.,1991; Harris and Hollstein 1993; Soussi 1995).

A mutation in the p53 suppressor gene is one of the most common genetic changes in different types of human cancer, even of the head and neck (Levine et al., 1994; Malkin 1994; Raybaud-Diogene et al., 1996; Zambetti and Levine 1993). This mutation leads to a protein antigenically identical to the wild-type but metabolically more stable, so with a longer half-life and characterized by altered regulatory properties for cell growth, resulting in the stimulation of cell proliferation.

Detection of p53 protein by immunohistochemical techniques is based on the longer half-life of the mutated form as opposed to the wild type. Moreover, although the p53 immunohistochemical staining is a rapid and simple screening method, it is not as specific for evaluation of p53 mutation (Melhem et al., 1995). In fact, studies performed on p53 over-expression in various neoplastic pathologies using not only immunohistochemistry but also molecular biology techniques, suggest that not always the two methods give the same positive results (Battiflora 1994; Kennedy et al., 1994; Kusama et al., 1996). Such discrepancies underline the need for caution in affirming that p53 over-expression is due to gene mutation. It is possible that the p53 protein, when cells are exposed to DNA damaging agents (such as ultraviolet light, gamma-irradiations, genotoxic chemicals) becomes stabilized and increases in concentration, without a mutation of the corresponding gene (Fritsche et al., 1993; Kastan et al., 1991; Maltzmann and Czyzyk 1984; Smith et al., 1995). In any case, the over-expression of p53 protein is frequently present in tumors and can be considered as a biomarker of greater aggressiveness (Ahomadeghe et al., 1995).

In this study we have observed a p53 over-expression in all Nps examined. This protein resulted in a significantly higher proliferative activity. In fact, all 15 cases with a PCNA index higher than the mean value are characterized by a strong staining of the p53 protein.

We can therefore maintain that a high PCNA-LI in lining epithelium of Nps is not only determined, as suggested by Coste et al., (1996 a,b,c), by growth factors, namely insuline-like growth factor (IGF-I), transforming growth factor β (TGF-β) and platelet-derived growth factor (PDGF), produced either by inflammatory cells or by the epithelial cells themselves, but also by the increase in p53 protein.

Therefore, the p53 over-expression, although a tumor characteristic, can be observed even in non-neoplastic tissues. Ahomadegbe et al., (1995) affirms that the presence of this protein in normal epithelium and in non-invasive lesions of the head and neck, may represent an early event in the multistep process of epithelial cell carcinogenesis.

Our results suggest that p53 over-expression plays an important role in the pathogenesis of Nps and could represent the first stage which occour in the respiratory epithelium in areas where Nps are formed. Its association with a high PCNA-index confers a more biological aggressiveness to these neoformations.

By using immunohistochemical determination of PCNA and p53, it should thus be possible to easily obtain accurate information on the biological pattern of each single case of polyposis and thus identify the more aggressive lesions, precisely those that tend to recurr even after accurate surgical removal and that require more rigorous controls.

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