Expression of Delta Np63 in Nasopharygeal Carcinoma and its significance

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Abstract **Objective** To study the expression and significance of delta Np63 in naso-phayngeal carcinoma NPC . **Methods** Sixty cases of nasopharyngeal biopsy speciments were studied using immunohistochemical technique to determine the expression of one of the p53 gene family member-delta Np63 isoform, and compared with 37 cancer nearby mucosa and 30 chronic mucositis of the nasopharynx. **Results** All 60 NPC tissues showed delta Np63 positive reaction. Among them, 3 cases of keratinizing squamous cell carcinoma (100%) were weakly positive, 66.67% of non-keratinizing carcinomas (38/57) and 83.33% of undifferentiated carcinomas (10/12) were strongly positive reaction. Chronic mucositiss and the mucosa nearby cancer showed positive reaction only at their basal and suprabasal layers. **Conclusion** Delta Np63 was found to be closely associated with kera-tocytes malignant hyperplasia, the more infantile or anaplastic the more strongly reaction. Delta Np63 is a valuable diagnostic marker for anaplastic squamous cell.

Key Words Nasopharyngeal carcinoma; Delta Np63; Immunohistochemistry

P63 is a recently identified member of p53 gene family. It is a gene essential for development of the sqamous epithelia and other stratified epithelia. Because of two promoters, p63 gene is transcribed into two main isoforms: one contain a transactivation domain that is TA p63 isoform and the other lack a transactivation domain, a truncated Delta Np63 isoform with a N-terminally deleted. TA p63 and Delta Np63 isoform have functionally different roles during epithemal development[1]. TA p63 switch for initiation of epithelial stratification and induces proliferation and inhibits terminal differentiation, while Delta Np63 isoform acts as a dominant-negative protein and allows basal keratinocytes to withdraw from the cell cycle and commit to terminal differentiation. P63 is essential for ectodermal differentiation, so that p63 knockout mice exhibit severe developmental abnormalities that mice lack squamous and other stratified epithelia, all their epithelia remain single layered [2,3]. So that Delta Np63 is highly expressed in embryonic ectoderm. Delta Np63 located in the nuclei of basal and suprabasal regenerative cells of sqamous epithelial tissues and in many squamous cell carcinomas. Nylander et al[4] showed that Delta Np63 is the predominant isoform in cell lines from squamous cell carcinoma of the head and neck. Here we characterize the expression of Delta Np63 in nasopharyngeal carcinoma (NPC).

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MATERIALS AND METHODS

Materials

A total of 60 cases of NPC were collected from the Department of Pathology, Tumor Hospital of Liuzhou (Guangxi Zhuang Autonomous Region)., from January 2000 to December 2001. All the NPC cases were confirmed by pathological diagnosis. Patients were 39 men and 21 women, and their age ranged from 26 to 70 (mean age of 46) years old.

According to WHO (1991) classification, sixty NPC were classified into keratinizing squamous cell carcinoma (KSCC) and nonkeratinizing carcinoma (NKC), the later subdivided into differentiated nonkeratinizing squamous cell carcinoma (DNKSCC) and undifferentiated carcinoma (UDC). In the present 60 cases, there were 3 KSCC and 57 NKC. Among the later, there were subdivided into 45 DNSCC and 12 UDC (7 large round cell carcino-

mas and 5 small cell UDC). Thirty-nine NPC cases (65%) were found cervical lymph node metastasis. 30 cases of chronic nasopharyngeal mucositis was sampled randomly for control. Here we defined that 3mm distances to the border of malignant cell nest of NPC as nearby epithelia (NE). There were 37 biopsy specimens with NE, including 7 with atypical proliferation and the remain 23 were normal columnal or/and squamous cells, parts of them hyperplasia in layers but not atypical.

Methods

The tissue specimens were fixed in 10% formaldehyde solution and paraffin embedded. Tissue blocks were cut into 4 um series sections, then de-waxed and soaked in 95% ethanol. After blocking the activity of endogenous peroxidase with 1% H_2O_2, antigenic retrieval using microwave heating in 0.01 mol/L citrate buffer was performed. Immunohistochemical technique adopted PV-9000 two-steps method (Beijing Zhongshan Bio-reagent Com.). Purified anti-delta Np63 monoclonal antibody was from Santa Cruz Com. USA (products No 8431), 1: 400 diluted in concentration.

Grades of immuno-staining

Delta Np63 is expressed in the nucleus of squamous cell. Only nucleus immuno-staining is regarded as positive. Depending on the percentage of cells showing nucleus-bound positive immunohistochemical staining, the results were divided into 4 grades as follows: negative < 5%; weakly positive, <25%; positive, 25% to 50%; and strongly positive > 50%. Five random fields per specimen were reviewed and classified by two independent pathologists using a light microscope at ×400 magnification.

Statistical analysis

The experimental results were calculated by X^2 test. Difference was considered significant when the P value was less than 0.05.

RESULTS

Expression of Delta Np63 in NPC, NE and chronic nasopharyngeal mucositis

Delta Np63 positive expression in NPC, nearby mucosas and chronic NP mucositis were shown in table 1. In the positive cells, their positive signal detected under microscope was shown yellow or brown in color that located in the nucleus, with a clean background. In the control groups, the expression of Delta Np63 in NP mucositis and NE limited at basal or/and supra-basal layers (Fig.1). In the normally proliferated and atypically proliferated epithelia, the positive cells increase in more layers (Fig.2). Positive cells sparsely distributed in squamous cell layer and granular cell layer but none in horny layer. In the NPC, cancerous cell nests were strongly positive expression. Among the three groups of NPC mentioned positive rates was very significant (X^2=82.49, P<0.01). NPC cell nest were compared to nearby epithelia and the chronic NP mucositis, the differences were very significant in statisties (t=11.77, P<0.01 and t=11.50, P<0.01 respectively). But there was no significant difference between NE and chronic NP mucositis (t=0.45, P> 0.05).

Expression of Delta Np63 between various types of NPC

The expression of delta Np63 in keratinizing sqamous cell carcinoma, differentiated nonkeratinizing squamous cell carcinoma and undifferentiated NPC was shown in table 2. Three cases of KSCC all showed weakly positive reaction, in one case positive cells surrounded the carcinoma cell nest while positive cells decreased in number inside the nests (Fig.3), the other 2 cases where weakly staining positive cells separately distributed among the negative carcinoma cells in the nest (Fig.4). In NKSCC, where positive cells showed diffuse or in large sheet distribution, sometimes the positive cells occupied the entire cell nest (Fig. 5). In the UDC strongly positive expression significantly increased in intensity. Statistical analysis was performed to compare the differences of the 3 groups of NPC, which showed very significant (X^2=12.53, P<0,01). KSCC compared with DNKSCC or UDC showed very significances (t=2.94, P<0.01, and t=3.33, P< 0.01 respectively), while DNKSCC compared with UDC appeared no significant difference.

Expression of Delta Np63 in metastatic NPC

The expression of delta Np63 in cervical lymph nodes metastasis and non-metastasis NPC was shown in table 3. There was no significant difference between the two groups (t=0.13, P>0.05).
In addition, delta Np63 expressed negatively in all adenocytes. Positively expressed in the basal and supra-basal layers of the squamouis metaplasia in

nasopharyngeal recessus, where positive cells increased in number in the area of epithelia proliferation but negative in the surface cell of the lumen (Fig.6). The malignant squamous cells of cervical adeno-squamous carcinoma of uterus acted as control were positive, while closely glandular cancer cells were negative for the immuno–staining (Fig.7).

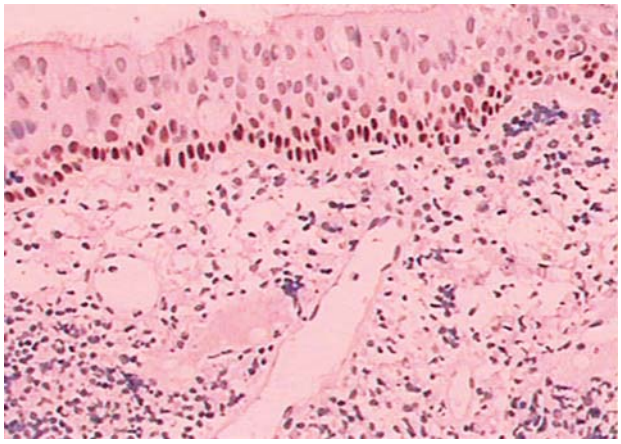

Fig.1 Nasopharyngeal mucous membrane are positive stained only in cells of the basement and superbasement layer.

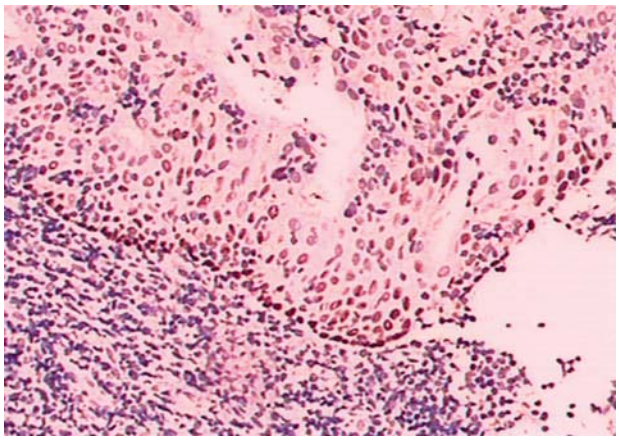

Fig.2 Delta NP63 protein expression increased in gradations in atypical hyperplastic mucous

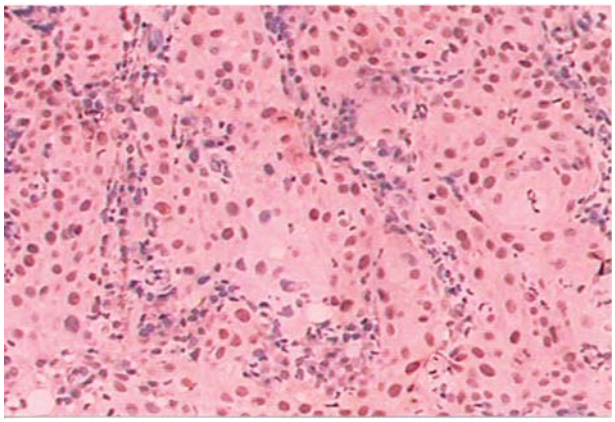

Fig.3 Keratinizing squamous cell carcinoma the edge cells of nest are delta NP63 positve stained which enrolled nest, While the cells in the center of nest are light or negative

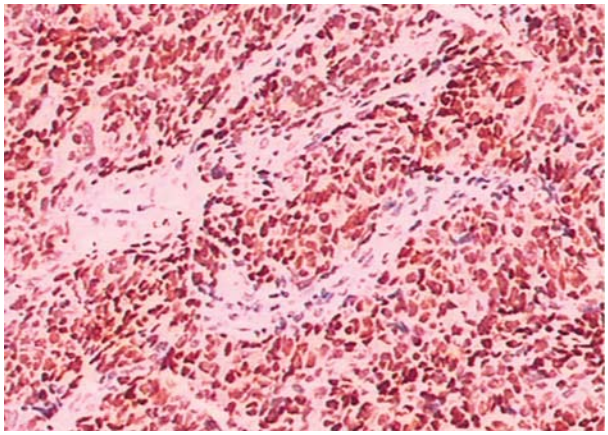

Fig.4 Nonkeratinizing carcinoma delta NP63 stained diffusely in large quantities

Tab.1 The Expression of delta Np63 in nasopharyngeal carcinoma, Nearby Mucos Membranes(MM) and Chronic Inflammation Tissues

Groups	Positive No	+ %	++ %	+++ %
NPC	60	5 8.33	17 28.33	38 63.33
Nearby MM	37	30 81.08	7 18.91	0 0.00
Chronic infl	30	26 80.00	4 20.00	0 0.00

Tab.2 The Expression of delta Np63 in Keratinizing, Nonkeratinizing and Undifferented Nasopharyngeal Carcinoma.

Groups	Positive No	+ %	++ %	+++ %
Keratin	3	3 100	0 00.00	0 0.00
Differented Nonkeratin	45	2(4.44)	15 33.33	28 62.22
Undifferent	12	0(00.00)	2(16.67)	10(83.33)

Tab.3 The Expression of delta Np63 Positive in Metastasis and Nonmetastasis Nasopharyngeal Carcinoma.

Groups	No.	+ %	++ %	+++ %
Nonmetastasis	21	3(14.28)	4(19.05)	14 66.67
Metastasis	39	2(5.13)	13 33.33	24 61.54

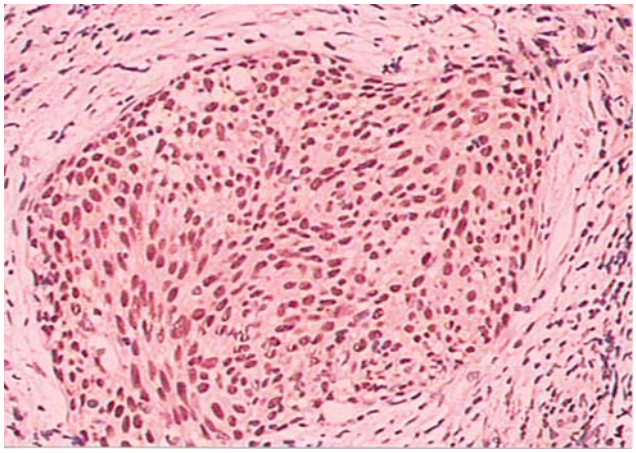

Fig.5 Nonkeratinizing carcinoma nucleus of the whole cancer nest are delta NP63 stained positive

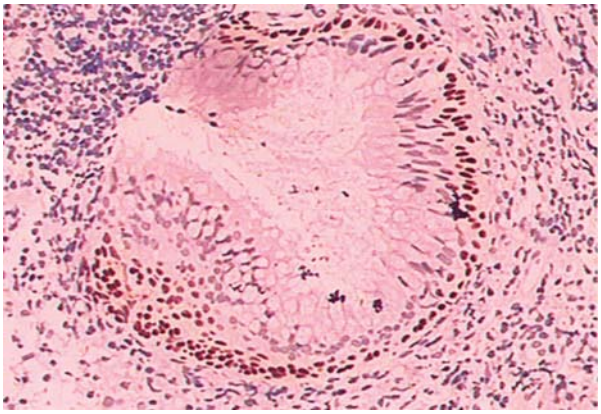

Fig.6 Basocells are delta NP63 positively stained in squamou cell metoplastic crypts, in the epithelial hyperplastic area positive expression increased in\ their gradations

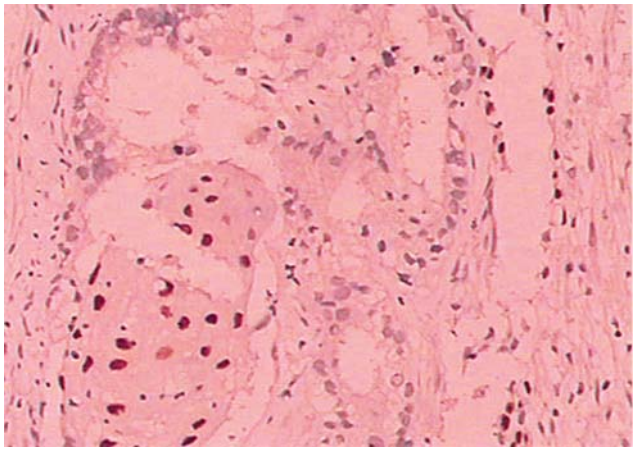

Fig.7 squamou cancer cells of the cervical adenosqua– mous carcinoma are ΔNP63 posiyive stained, the nearby glandular cancer cells are negative

DISCUSSION

Nonkeratinizing carcinoma constitute the majority form of nasopharyngeal carcinoma, the malignant cells are low-differentiation and polymorphism. Tumor suppressor gene p53 rarely has mutations in NPC [5,6]. However, using immunohistochemical method can detect high expression of p53 protein in NPC tissues[7]. It is known that wild type p53 is not detectable by immunohistochemical method, because of very short half-life. High expression of P53 in NPC suggests its inactivation in function. The present study analyzes the delta Np63 with related and antagonistic properties towards p53 [8] in NPC. Delta Np63 is required for the differentiation of normal squamous cell and expressed in both non-neoplastic squamous epithelium and squamous cell carcinoma of the nasopharynx. On condition that the epithelia situated in chronic inflammation or close-in carcinoma nests, the distribution of Delta Np63 was restricted to the proliferating basal and suprabasal layers. If there is any atypical hyperplsia, the expressed layers will be increased. The expressing rate of delta Np63 is up to 100% in all the 60 cases of NPC tissues, which indicating all the cases virtually differentiate in varying grades including UDC form. Moreover, inside NPC the number of cells containing delta Np63 and their distribution depends on the degree of anaplsia. In highly differentiated KSCC form, delta Np63 is confined to a ring of basal like cells surrounding, whereas weaker and finally negatively staining are observed toward the central area with keratin pearl formation. There is more strongly expression in NKSCC than in KSCC. The most strongly expression is observed in UDC where almost all the

anaplastic cells are containing delta Np63 and showing deeper in color. The percentage of positive cells progressively increases from normal to neoplastic cell, and to UDC. Since p63 can block p53 mediate transactivation, it is potentially a dominant negative isoform. P53 and delta Np63 being high expression in anaplastic NPC cells indicated that the wild type p53 protein is inactivated by p63 through a delta Np63 pathway[9], which might be a reasonable explanation for this phenomenon. In any case, delta Np63 shows to be a valuable maker for anaplastic keratinocytes, and a valuable diagnostic marker for NKC of the NPC[10].

P63 is essential for several aspects of ectodermal differentiation during embryogenesis. The skin of p63 deficient mice does not progress past the early development stages [11], their progenitor cell could not develop to normal squamous epithelium. Also in the present study we identify that positive expression of delta Np63 protein was observed only in the proliferating cells towards squamous epithelia and negative toward adenocytes. This observation was not only in NPC but also observed in cervical carcinoma where squamous cell carcinoma p63 protein positive while nearby adeno-carcinoma cells negative, Previously, pathologist depend on tonofilament and desmosome only to distinguish anaplastic squamous cell carcinoma from other originally undifferentiated carcinomas. However, some of the undifferentiated cell have little cytoplasm and nor clear boundary that limits these distinguishing ability. While delta Np63 situated on the nucleus does not depend on cytoplasm, it can be a good marker for diagnosis of undifferentiated squamous cells carcinoma and adeno-squamous carcinoma.

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