

OVEREXPRESSION OF P53 AND NEOPLASTIC CELL PROLIFERATION IN UNDIFFERENTIATED NASOPHARYNGEAL CARCINOMA

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Abstract. The author investigated the p53 status in correlation with cellular proliferation in the undifferentiated subgroup, which is infrequently found in caucasians. The author evaluated formalin-fixed, paraffin embedded tissue blocks from sixty cases with undifferentiated carcinoma of the nasopharynx by p53 and Ki67 immunostaining. All samples were retrieved from the surgical pathology file at King Chulalongkorn Memorial Hospital from 2001-2005. The patients had a mean age of 47 years. Stage IV was the most common stage, found in 21 cases (35%). Forty-four tumors (73%) overexpressed p53 protein, which was significantly associated with high rate of tumor cell proliferation ($r = 0.477$, $p < 0.001$). The higher the amount of p53 stained, the higher the rate of tumor cell proliferation. However, there was no statistically significant association between p53 protein overexpression and clinical status, including tumor volume, nodal status, and metastatic condition. This observation may explain why some tumors are resistant to radiation and are poorly controlled when they recur in distant organs.

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a highly malignant neoplasm, arising from the nasopharyngeal epithelium. It is found occasionally in many geographic areas, although the incidence is highest among Southern Chinese and the population in Southeast Asia (Liu *et al*, 2002; M'Rabti *et al*, 2006). There is evidence that Epstein-Barr virus infection plays an initial role. Interaction between environmental and genetic factors is essential for tumor phenotype, growth, and development (Jayasurya *et al*, 2000; Chan *et al*, 2002). According to current concepts of tumor genetics, activation of proto-oncogenes, inactivation of tumor-suppressor genes, or both are

thought to be indispensable for human carcinogenesis. These genetic aberrations are certainly associated with NPC (Burgos, 2005).

Regarding tumor-suppressor genes, *p53* is the most common genetic alteration in various human malignancies including some fractions of NPC (Chen *et al*, 2004; Cheng *et al*, 2005). This gene is located on the short arm of chromosome 17 which expresses a high level of protein products responsible for DNA damage (Baker *et al*, 1989). The p53 protein changes its structure to attach to damaged DNA in order to cease the cell cycle, particularly late G1 and G2 phases, and allows enough time for DNA repair. After repair, cell cycle activity is permitted to proceed and p53 protein is degraded without intranuclear accumulation. However, if DNA repair does not succeed, it will be demolished by proapoptotic genes under p53 protein regulation (Buschmann *et al*, 2000; Iacopetta, 2003). In contrast, the mutated *p53* gene overproduces abnormal p53 protein which has an abnormal

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function. Excessive amounts of this protein are not destroyed and fail to induce DNA repair or regulate the apoptotic process (Lopez-Crapez *et al*, 2005).

The past decade has witnessed an important shift in patient management. Histological classification and advances of the knowledge of the biology of NPC have had major prognostic significance, along with distance of tumor extension within the TNM staging system. Previous research from Western countries has suggested that non-keratinizing NPC is more responsive to radiotherapy due to the low incidence of p53 mutation, when compared to keratinizing NPC (Reddy *et al*, 1995; Marks *et al*, 1998). Those studies combined differentiated and undifferentiated subtypes because of the low incidence of NPC among caucasians. However some non-keratinizing tumors are radioresistant and have a high recurrence. The treatment does not succeed when NPC spreads to distant organs (Cheng *et al*, 2001; Chan *et al*, 2002; Shi *et al*, 2002; Johnson *et al*, 2005). To explicate some aspects of neoplastic cell biology, the author collected cases of undifferentiated NPC to evaluate the status and correlation between p53 protein and tumor cellular proliferation. These observations may be useful as a predictor of patient outcomes.

MATERIALS AND METHODS

Seventy-nine cases diagnosed with undifferentiated nasopharyngeal carcinoma between 2001 and 2005 were retrieved from the surgical pathology files at King Chulalongkorn Memorial Hospital. Tumor tissue was available for examination in 60 of these cases. The remaining nineteen cases were excluded from the study. Their histological features were reviewed on two-micrometer-thick hematoxylin and eosin stained slides.

The p53 (Dako, dilution 1:300) and Ki67 (Dako, dilution 1:300) immunostains were

used to investigate p53 overexpression and the nuclear proliferation index, respectively. The standard direct streptavidin-biotin immunohistochemical technique was used. Chromogen diaminobenzidine tetrahydrochloride (DAB) was used as a detection agent on the tumor tissue sections. Both stains were semiquantitatively evaluated. Positive cells were those expressing nuclear brown stain. Field selection used the highest p53 and Ki67 expression areas (hot spots) on low power scanning then divided into five groups: <10% was negative, 10-25% was 1+, 26-50% was 2+, 51-75% was 3+, and >75% was 4+.

Data were analyzed by SPSS for Windows, Version 11.5. The correlation between p53 and Ki67 immunohistochemistry was calculated by means of the Spearman's rank correlation coefficient. Confidence intervals were 95%. A p-value of <0.05 was considered statistically significant.

RESULTS

The sixty subjects consisted of 45 males (75%) and 15 females (25%) with a mean age of 47 years (SD=13.7). According to TNM staging of head and neck tumors by the World Health Organization (Chan *et al*, 2005), 8 cases (13%) were stage I, 12 cases (20%) were stage II, 19 cases (32%) were stage III, and 21 cases (35%) were stage IV.

The frequency and distribution of tumors according to p53 and Ki67 immunostainings are shown in Table 1. The numbers of tumors negative and positive for p53 were 16 (27%) and 44 (73%) cases, respectively. For Ki67, 25 cases (42%) were negative immunohistochemically whereas 35 cases (58%) were positive as seen in Fig 1. Moreover the correlation between p53 overexpression and tumor nuclear proliferation, detected by Ki67, was statistically significant ($r = 0.477$, $p < 0.001$). In another words, the more positive the p53 was, the higher the proliferation rate.

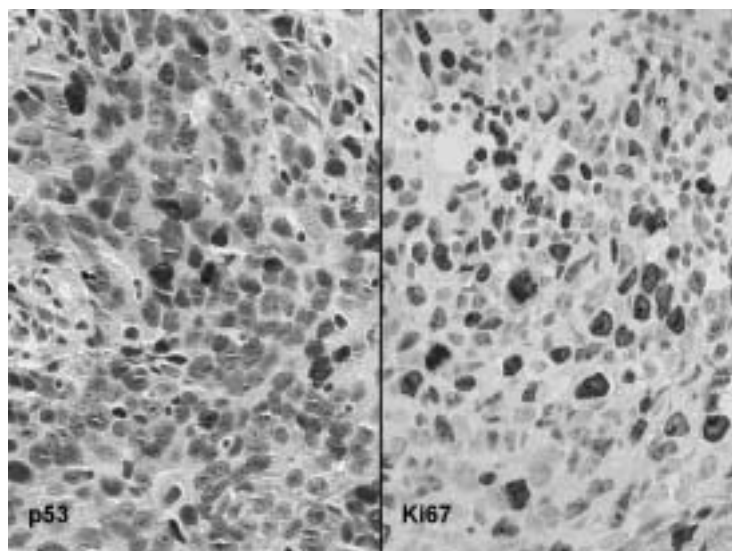


Fig 1—Undifferentiated nasopharyngeal carcinoma with positive nuclear immunostaining of p53 (left) and Ki67 (right).

Table 1
Distribution of p53 and Ki67
immunohistochemistry in 60 patients with
undifferentiated nasopharyngeal carcinoma.

	p53 No. (%)	Ki67 No. (%)
Negative	16 (27)	25 (42)
Positive 1+	4 (7)	16 (27)
Positive 2+	10 (17)	5 (8)
Positive 3+	7 (11)	3 (5)
Positive 4+	23 (38)	11 (18)

DISCUSSION

Approximately 97% of malignant tumors originating from the nasopharynx are NPC, the pathogenesis of which is related to EBV infection as well as incorporation of genetic and environmental factors (Chan *et al*, 2002; Liu *et al*, 2002). The World Health Organization classifies NPC into three categories: keratinizing, non-keratinizing, and basaloid squamous cell carcinoma (Chan *et al*, 2005). In endemic areas, such as Thailand, the prevalence of the

non-keratinizing group is high and can be subclassified into differentiated and undifferentiated subtypes (Chan *et al*, 2005; M'Rabti *et al*, 2006).

TNM staging and histological confirmation are not enough for patient management. A knowledge of tumor biology is needed for developing new treatment strategies. The tumors with *p53* mutation tend to be resistant to ionizing radiation and chemotherapeutic drugs. Non-keratinizing NPCs are more responsive to radiotherapy due to a lower incidence of

p53 mutations. However, those studies were published in Western countries where non-keratinizing NPC occurs sporadically. They do not distinguish between differentiated and undifferentiated subtypes (Reddy *et al*, 1995; Sanguineti *et al*, 1997; Marks *et al*, 1998). The author selected only the undifferentiated subtype in Thai people for determining the *p53* status and nuclear proliferation rate, along with the correlation between them.

According to this study, 73% of undifferentiated NPC cases overexpressed *p53* protein. The *p53* status was significantly correlated with the rate of tumor cell proliferation. Although *p53* protein was produced excessively by neoplastic cells, its function was altered. This protein loses the ability to enhance apoptosis following phosphorylation. Thus, the abnormally rapid malignant cell cycle still carries on and neoplastic cells become immortal, as demonstrated by Ki67 immunohistochemistry (Buschmann *et al*, 2000; Iacopetta, 2003; Agaoglu *et al*, 2004; Lopez-Crapez *et al*, 2005). Moreover, tumor cells which with a mutated *p53* gene do not re-

spond to radiation or chemotherapy. Both treatment modalities act as external stimuli, leading to DNA injury, but the malignant cells are not demolished by the apoptotic pathway due to dysfunction of the mutated p53 protein (Baker *et al*, 1989; Buschmann *et al*, 2000; Iacopetta, 2003). Masuda *et al* (1998) found resistance to radiotherapy and a poor prognosis in NPC patients with p53 mutation. Normal cell cycle fluctuation harboring wild-type p53 may give a low percentage of p53 protein expression and the immunohistochemical stain cannot distinguish this condition from an oncogenic state. As with a previous report, the author used 10% nuclear staining as a cut off point (Lopez-Crapez *et al*, 2005). For proliferation activity assessment, antibody directed against the Ki67 antigen is more accurate than counting mitoses or proliferating cell nuclear antigen (PCNA) staining. Mitoses are easily identified in only a small proportion of the cell cycle, whereas the half-life of the PCNA antigen exceeds the cell cycle time (Harn *et al*, 1998; Lopez-Crapez *et al*, 2005).

Despite the high incidence of p53 overexpression, it was not associated with TNM stage in this study. Chen *et al* (2004) reported that tumor volume was larger in those with high level of p53 on immunostains. Cheng *et al* (2005) found a correlation between p53 gene expression and cervical lymph node metastasis. However, both studies were conducted on all types of NPC. Although the duration of follow-up was too short to demonstrate clinical significance, this study revealed a high incidence of p53 protein overexpression in undifferentiated NPC, strongly correlated with cellular proliferation. This may result in radioresistance in some non-keratinizing NPC cases, especially in the undifferentiated subgroup.

In summary, nearly three-fourths of undifferentiated NPC patient in this study had p53 protein overexpression and which was significantly associated with the degree of tumor cell proliferation identified by Ki67 immu-

nohistochemistry.

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