Increased PA2G4 Expression Is an Unfavorable Factor in Nasopharyngeal Carcinoma

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Abstract: PA2G4 plays a dual role in tumors. However, the correlation of its expression with clinical feature and prognosis has never been reported in nasopharyngeal carcinoma (NPC). Using immunohistochemical staining, we examined PA2G4 protein level in clinicopathologically characterized 201 NPC cases (138 male and 63 female) with age ranging from 21 to 83 years and 45 nasopharyngeal (NP) tissues. Statistical methods were used to assess the difference in PA2G4 expression and its relationship with clinical parameters and prognosis in NPC. Immunohistochemical analysis showed that the protein expression of PA2G4 examined in NPC tissues was higher than that in the nasopharyngeal tissues \((P=0.005)\). In addition, high levels of PA2G4 protein were positively correlated with tumor size \((T\text{ classification}) \,(P<0.001)\), the status of lymph node metastasis \((N\text{ classification}) \,(P<0.001)\), distant metastasis \((P=0.029)\), and clinical stage \((P<0.001)\) of NPC patients. Patients with higher PA2G4 expression had a significantly shorter overall survival time than did patients with low PA2G4 expression. Stratified analysis indicated that high expression of PA2G4 showed the inverted survival time in clinical stages III-IV, but not stages I-II. Finally, multivariate analysis suggested that the level of PA2G4 expression was an independent prognostic indicator \((P<0.001)\) for the survival of patients with NPC. Elevated protein expression of PA2G4 was significantly shown, which plays an unfavorable outcome for NPC patient survival.

Key Words: nasopharyngeal carcinoma, PA2G4, prognosis, immunohistochemistry

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Two hundred one NPC specimens and 45 noncancerous nasopharyngeal (NP) tissues. Furthermore, the relatively higher protein expression of PA2G4 was associated with NPC progression and poor prognosis. Our results demonstrated that elevated PA2G4 protein level as an independent prognostic factor plays an unfavorable prognostic factor for NPC patient’s survival.

MATERIALS AND METHODS

Microarray Data Analysis

In previous studies, Fang et al.\(^1\) had reported to use microarray to examine the differential expression genes in NPC tissues or NPC cells compared with NP tissues. In this investigation, we reanalyzed the differential genes of NPC compared with NP based on the data provided by Fang et al.\(^1\)

Sample Collection

Two hundred one NPC specimens and 45 noncancerous nasopharynx paraffin-embedded specimens were obtained...
from Nanfang Hospital, Guangzhou, China. In these cases, there were 138 male and 63 female with age ranging from 21 to 83 years. For the use of these clinical materials for investigation purposes, prior consents from the patients and approval from the Ethics Committees of Nanfang Hospital were obtained. All specimens had proved pathologic diagnosis and were staged according to NPC staging system of the WHO in 2009.

**Immunohistochemistry**

Immunohistochemical staining was performed according to the previous descriptions. NPC and NP specimen paraffin sections were deparaffinized in 100% xylene and rehydrated in descending ethanol series according to standard protocols. Heat-induced antigen retrieval was performed in 10 mM citrate buffer for 2 minutes at 100°C. Endogenous peroxidase activity and nonspecific antigen were blocked with peroxidase blocking reagent containing 3% hydrogen peroxide and serum, followed by incubation with mouse PA2G4 antibody (1:100) (Proteintech, Wuhan, China) for 1 hour at 37°C. After washing, the sections were incubated with biotin-labeled rabbit anti-goat antibody for 10 minutes at room temperature, and subsequently were incubated using streptavidin-conjugated horseradish peroxidase (HRP) (Maixin Inc, China). Sections

![FIGURE 1](image1.png)

**FIGURE 1.** PA2G4 messenger RNA is upregulated in nasopharyngeal carcinoma (NPC) tissues or NPC cells compared with nasopharyngeal tissues based on the microarray data analysis.

![FIGURE 2](image2.png)

**FIGURE 2.** PA2G4 expression in NPC and NP tissues. Specific PA2G4 protein expression was observed in the cytoplasm of the malignant tumor cells and full thickness nasopharyngeal mucosa epithelium, including cilia. A, Low expression of PA2G4 in NP tissues (×400). B, High expression of PA2G4 in NP tissues (×400). C and D, Low expression of PA2G4 in NPC tissues (×400). E and F, High expression of PA2G4 in NPC tissues (×400). G, Low expression of PA2G4 in NP tissue and high expression in NPC tissues in the same section (×100). NP indicates nasopharyngeal; NPC, nasopharyngeal carcinoma.
were visualized with DAB and counterstained with hematoxylin, mounted in neutral gum, and analyzed using a bright field microscope.

**Evaluation of Staining**

The immunohistochemically stained tissue sections were reviewed and scored separately by 2 pathologists blinded to the clinical parameters. The staining score was previously described according to the sum of staining intensity and the percentage of positive staining areas (0 to 7). For statistical analysis, a final staining scores of 0 to 5 and 6 to 7 were, respectively, considered to be low and high expression.

**Statistical Analyses**

All statistical analyses were carried out using SPSS 20.0 software. Data were shown as mean ± SD. The χ² test was explored to analyze the association between the levels of PA2G4 expression and clinicopathologic characteristics. Survival curves were plotted by the Kaplan-Meier assay and compared using the log-rank test. The significances of various variables in survival were analyzed using multivariate Cox proportional hazards model. A P-value of <0.05 was considered to be statistically significant.

**RESULTS**

On the basis of the analysis of microarray data, we observed that PA2G4 level was significantly increased in pooled NPC cells and 8 NPC pooled tissues compared with NP tissues (Fig. 1).

Then we measured the expression levels and subcellular localization of PA2G4 protein in 201 paraffin-embedded NPC specimens and 45 noncancerous NP samples using immunohistochemical staining assay. Specific PA2G4 protein expression was observed in the cytoplasm of the malignant tumor cells and full thickness NP mucosa epithelium, including cilia (Figs. 2A–G). Furthermore, we observed that in 50.2% (101/201) of NPC samples, PA2G4 protein level was elevated. In comparison, 26.7% (12/45) of noncancerous NP samples had increased PA2G4 protein level, significantly lower than that in the NPC samples (P = 0.005) (Table 1).

We next analyzed the relationships between clinicopathologic characteristics and PA2G4 expression levels in individuals with NPC (Table 2). Although we did not find a significant association of PA2G4 expression levels with patient’s age, sex, and smoking, we observed that the expression level of PA2G4 was positively correlated with tumor size (T classification) (P < 0.001), the status of lymph node metastasis (N classification) (N0-N1 vs. N2-N3) (P < 0.001) and status of distant metastasis (M classification) (P < 0.001) in 201 NPC patients (Table 2).

To investigate the prognostic value of PA2G4 expression for NPC, we further assessed the association between the levels of PA2G4 expression and patients’ survival using Kaplan-Meier analysis with the log-rank test. In 201 NPC cases with prognosis information, we observed that the level of PA2G4 expression was significantly correlated with the overall survival of NPC patients (Fig. 3A). Patients with high level of PA2G4 expression had poorer survival than those with lower level of PA2G4 expression (P = 0.001). We also observed that higher tumor PA2G4 protein expression was associated with a shorter survival time for patients in clinical stage III-IV (Fig. 3C) (P = 0.010, but not I-II (P = 0.821) (Fig. 3B).

Univariate assay showed that PA2G4 expression, T, N, M classification, and clinical stage were correlated significantly with patient survival (P < 0.001, P < 0.001, P < 0.001, P < 0.001, and P < 0.001, respectively). A multivariate analysis of PA2G4 protein expression levels adjusted for T, N, M classification, and clinical stage showed that the level of PA2G4 expression was an independent prognostic factor for NPC (P < 0.001; Table 3).

**DISCUSSION**

The PA2G4 gene belongs to a member of the described PA2G4 family. The mouse p38-G4 gene is the prototype of this family, which was isolated by generating monoclonal antibodies against DNA binding proteins. The sequence of the human PA2G4 cDNA encodes a 394 amino acid protein and is ~45 kDa molecular weight. It
has a longer N-terminal region than mouse p38-2G4 protein. In previous studies, PA2G4 might play a role in an ERBB3-regulated signal transduction pathway. It was shown to be interacted with ERBB3 and promoted or suppressed the tumor pathogenesis. Furthermore, PA2G4 seems to be involved in growth regulation and acts a corepressor of the androgen receptor and is modulated by the ERBB3 ligand neuregulin-1/heregulin (HRG). However, the role of PA2G4 was still unclear in NPC.

PA2G4 has reported to be overexpressed in some tumors including salivary adenoid cystic carcinoma, brain tumor, pancreatic ductal adenocarcinoma, acute myelogenous leukemic cells, cervical cancer, oral cancer. However, inversed data showed its downregulated expression in breast cancer, bladder cancer, prostate cancer, which hinted a dual role in tumors. In previous study, Xiao et al found that PA2G4 was upregulated in NPC compared with NP and serum samples based on proteomics assay. Here, we firstly analyzed the microarray data provided by Prof Fang and observed that PA2G4 was obviously increased in NPC tissues or NPC cells compared with NP tissues. Further, we used immunohistochemistry staining to examine the expression of PA2G4 in NPC and NP tissues. The data showed that PA2G4 protein expression was significantly upregulated in NPC. This data supported Xiao and colleagues’ data, which suggested that elevated expression of PA2G4 promoted the pathogenesis of NPC.

In previous documents, overexpression of PA2G4 was reported to correlate with the clinical progression of PA2G4 in tumors. In this study, we observed that...
TABLE 3. Summary of Univariate and Multivariate Cox Regression Analysis of Overall Survival Duration

| Parameter                | Univariate Analysis | 95% CI       | Multivariate Analysis | 95% CI       |
|--------------------------|---------------------|--------------|-----------------------|--------------|
| Sex                      | P                    | HR           |                       | HR           |
| Male vs. female          | 0.324               | 1.265        | 0.792-2.020           |              |
| Age (y)                  |                     |              |                       |              |
| ≥ 50 vs. <50             | 0.384               | 1.203        | 0.794-1.823           |              |
| Smoking                  |                     |              |                       |              |
| Yes vs. no               | 0.936               | 1.024        | 0.578-1.814           |              |
| T classification         |                     |              |                       |              |
| T1-T2 vs. T3-T4          | 0.000               | 3.303        | 2.154-5.064           | 0.011        | 1.949        | 1.165-3.261 |
| N classification         |                     |              |                       |              |
| N0-N1 vs. N2-N3          | 0.000               | 3.350        | 2.156-5.207           | 0.032        | 1.871        | 1.056-3.315 |
| M classification         |                     |              |                       |              |
| M0 vs. M1                | 0.000               | 5.402        | 2.973-9.817           | 0.001        | 2.908        | 1.564-5.408 |
| Clinical stage           |                     |              |                       |              |
| I-II vs. III-IV          | 0.000               | 7.030        | 3.707-13.334          | 0.026        | 2.657        | 1.123-6.285 |
| PA2G4 level              |                     |              |                       |              |
| High expression vs. low expression | 0.000 | 2.741 | 1.751-4.291 | 0.000 | 0.239 | 0.125-0.458 |

CI indicates confidence interval; HR, hazard ratio.

Although overexpressed PA2G4 was not associated with sex, age, and smoking, it positively correlated with T classification (tumor size), N classification (lymph node metastasis), M classification (distant metastasis), clinical stages of NPC patients. These data were similar to Mei et al’s report, which suggested that overexpressed PA2G4 significantly accelerated the pathogenesis of NPC and played an unfavorable role for NPC prognosis. However, the relationship between PA2G4 expression and the survival of NPC patients was still to be determined.

In prior documents, expression of PA2G4 in tumor cells has been indicated to be a favorable or unfavorable prognostic factor depending on tumor types. Hu et al found that decreased expression of PA2G4/EBP1 is a favorable factor in hepatocellular carcinoma. Interestingly, opposite results were reported in salivary adenoid cystic carcinoma. Sun et al showed that the higher expression of PA2G4 caused an unfavorable outcome.

In the present study, we provide the proof to demonstrate that elevated PA2G4 protein expression was inversely correlated with patient’s overall survival prognosis in NPC. The patients with higher expression of PA2G4 protein had shorter survival time. Stratified analysis further indicated that although overexpressed PA2G4 was not related to survival prognosis in clinical stage I and II, it indicated the worse survival prognosis level in clinical stage III and IV. The data indicated that the overexpression of PA2G4 could be used as a prognostic indicator for mid-advanced NPC, but not mid-early NPC.

Finally, we analyzed the possibility of PA2G4 as an independent prognostic factor. On the basis of multivariate analyses, elevated expression of PA2G4 protein was a significantly independent predictor of poor prognosis for NPC patients. These data further revealed the significance of overexpressed PA2G4 in NPC pathogenesis.

In summary, this study confirmed that the expression level of PA2G4 was significantly upregulated in NPC and correlated with the malignant progression of NPC. Furthermore, our data demonstrated that overexpression of PA2G4 was an unfavorable prognostic factor for NPC. Finally, PA2G4 expression level is an independent prognosis factor predicting NPC pathogenesis.

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