Clinical Research Paper

Expression and clinical significance of Wnt-1 and \( \beta \)-catenin in nasopharyngeal carcinoma

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Background and Objective: As signaling molecule and key component of Wnt/\( \beta \)-catenin signaling pathway respectively, Wnt-1 and \( \beta \)-catenin are abnormally expressed in several malignancies and correlate with poor prognosis. This study was to investigate the expression and clinical significance of Wnt-1 and \( \beta \)-catenin in nasopharyngeal carcinoma (NPC). Methods: The expression of Wnt-1 and \( \beta \)-catenin in 111 specimens of NPC was detected by SP immunohistochemistry. Their correlations to relapse-free survival (RFS), metastasis-free survival (MFS) and progression-free survival (PFS) were analyzed. Results: The high expression of \( \beta \)-catenin was observed in 64 (57.7%) of the 111 cases. Its high expression rate was significantly higher in advanced NPC than in early stage NPC (63.1% vs. 40.7%, \( p = 0.041 \)). The RFS, MFS and PFS were lower in high \( \beta \)-catenin expression group than in low \( \beta \)-catenin expression group (\( p < 0.05 \)). Cox regression analysis demonstrated that \( \beta \)-catenin was related to poor prognosis of NPC patients. The high expression of Wnt-1 was observed in 68 (61.3%) of the 111 cases, but its expression had no effect on RFS, MFS and PFS (\( p > 0.05 \)). Conclusions: Wnt/\( \beta \)-catenin signaling pathway may be activated abnormally in some NPC patients. \( \beta \)-catenin may be a prognostic factor of NPC.

Nasopharyngeal carcinoma (NPC) is a common malignancy in southern China. Its 5-year survival rate has been significantly improved in recent years, however, relapse and metastasis are still the main failure patterns. Clinical staging is currently the major measure to evaluate the prognosis of NPC, however, patients at the same stage may have different prognosis. Therefore, it’s necessary to explore reliable and effective molecular markers for executing individualized therapy, intensifying follow-up of high risk patients, and detecting any progression as early as possible.

Wnt/\( \beta \)-catenin signaling pathway can regulate stem cell proliferation and differentiation, which is recently considered to be associated with development and progression of tumor.1,2 Wnt-1, a signalling molecule in the pathway, can promote the genesis of many tumors,3,5 but its correlations to tumour progression and to the prognosis of NPC have seldom been reported. As a key signalling molecule, \( \beta \)-catenin is one of the poor prognostic factors for breast cancer6 and colorectal cancer,7 while its prognostic significance in NPC is still controversial and worthy of investigating. This study was to detect the expression of Wnt-1 and \( \beta \)-catenin by immunohistochemistry and investigate their relationship to the relapse and metastasis of NPC.

Materials and Methods

Patient data. Nasopharyngeal biopsy specimens from 111 NPC patients, who were initially treated in Sun Yat-sen University Cancer Center from January 1999 to December 1999, were collected. All patients had pathologically confirmed poorly differentiated squamous cancer or undifferentiated cancer without evidence of distant metastasis. Of the 111 patients, 87 were men and 24 were women, the median age was 47 years (range, 18–71 years); according to ’92 Fuzhou staging, three had stage I tumor, 24 had stage II tumor, 58 had stage III tumor, and 26 had stage IVa tumor. All patients received radical radiotherapy with \( ^{60} \)Co \( \gamma \) radiation or 6/8 MV linear accelerator. Conventional external radiation was applied to the facial-cervical joint portal in 108 patients and to the facial-cervical subportal in three patients. The total radiation dose to the nasopharynx was 68–80 Gy, while four patients received afterloading irradiation of 15–16 Gy following 60 Gy radiation and one received afterloading irradiation of 9 Gy following 70 Gy radiation. The therapeutic dose to the neck was 60–70 Gy and the preventative dose was 50–56 Gy. Twenty-four patients received inductive PF regimen-dominating chemotherapy, and ten received platinum-based simultaneous chemotherapy.

Reagents. Wnt-1 goat anti-human polyclonal antibody (sc-6280) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). \( \beta \)-catenin mouse anti-human monoclonal antibody (ZM-0442) and SP-9003 immunohistochemical staining kit were purchased from

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Zhongshan Golden Bridge Biotechnology (Beijing, China). The working concentration for concentrated Wnt-1 antibody was 1:200 and that for concentrated β-catenin antibody was 1: 100.

Immunohistochemistry. All paraffin-embedded tissues were successively sectioned at the thickness of 4 μm and routinely deparaffinized and rehydrated. Antigens were retrieved for 20 min using microwave and returned to room temperature. Subsequently, endogenous peroxidase was eliminated with PBS and non-specific antigenic determinants were blocked with rabbit serum. Sections were incubated with primary antibody at 4°C overnight, incubated with secondary antibody at 37°C for 30 min the next day, and further incubated with horseradish peroxidase-labeled streptavidin at room temperature for 20 min, then stained with DAB and counterstained with hematoxylin. PBS (0.01 mol/L) instead of primary antibody was used as negative control, while Wnt-1- and β-catenin-positive breast cancer tissues were used as positive control.

Results evaluation. Under microscope, ten visual fields were randomly examined and 100 cells in each visual field were counted. Positive signals of Wnt-1 located on cell membrane or in cytoplasm while those of β-catenin located in cytoplasm or nucleus as brownish granules. Staining intensity of positive signals and the proportion of positive cells were examined and scored respectively. No staining was scored as 0 point, light brownish staining as 1 point, brownish staining as 2 points, and heavy brownish staining as 3 points; a positive proportion of 1–24% was scored as 1 point, 25–49% as 2 points, 50–74% as 3 points, and 75–100% as 4 points. The two scores were multiplied to obtain the final score. According to the median of final scores, the specimens with final scores below the median were subjected to low expression group, and those with final scores equal or above the median were subjected to high expression group.

Follow-up. Complete clinical record and follow-up data of all patients were available. The median follow-up duration was 65 months (8–88 months). The 1-, 3- and 5-year follow-up rates were 98.2%, 97.3% and 93.7%, respectively.

Statistical Analysis. SPSS13.0 statistical software package was used to analyze the data. Chi-square test was used to analyze count data. Spearman test was used for correlation analysis. Life table and Kaplan-Meier method were used for survival analysis. Cox regression model was used for multivariate prognostic analysis. Log-rank test was used for intergroup comparison. p value of < 0.05 indicated significant difference. Recurrence-free survival (RFS) referred to the duration from the beginning of treatment to the occurrence of local recurrence.
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Results

Follow-up. During follow-up, the local-regional recurrence rate was 18.9% (21/111), the metastasis rate was 14.4% (16/111), and the overall survival rate was 62.2% (69/111).

Expression of Wnt-1 and β-catenin and their correlations. Wnt-1 and β-catenin were mostly expressed in cytoplasm of cancer cells (Fig. 1). Of the 111 patients, 68 (61.3%) had high expression of Wnt-1, while 64 (57.7%) had high expression of β-catenin. Wnt-1 expression was positively correlated to β-catenin expression (r = 0.387, p < 0.001).

Correlation of Wnt-1 and β-catenin expression to clinical characteristics. The expression of Wnt-1 had no relationships with sex, age, T stage, N stage, and 92’ TNM stage (p > 0.05). The high expression rate of β-catenin was significantly higher in the cases of advanced stage than in those of early stage (p = 0.041), but it had no relationships with sex, age, T stage, and N stage (p > 0.05) (Table 1).

Correlation of Wnt-1 expression to prognosis. The 5-year RFS, MFS and PFS rates were 84.4%, 87.5% and 82.4% in low Wnt-1 expression group, and were 76.7%, 73.1% and 61.6% in high Wnt-1 expression group (p > 0.05).

Correlation of β-catenin expression to prognosis. The 5-year RFS, MFS and PFS rates were 88.4%, 93.6% and 82.3% in low β-catenin expression group, and were 73.0%, 77.2% and 54.0% in high β-catenin expression group (p < 0.05) (Fig. 2–4).

Univariate and multivariate analysis of progression-free survival. A total of 11 factors, including sex, age, 92 stage, T stage, N stage, nasopharyngeal dose, cervical lymph node dose, inductive chemotherapy, simultaneous chemotherapy, Wnt-1 expression and β-catenin expression, were introduced into univariate analysis of PFS, revealing that only β-catenin expression and 92 stage were related with PFS (p < 0.05). Sex, age, 92 stage, T stage, N stage and β-catenin expression were further introduced into Cox regression model using the Enter method, revealing that only sex and β-catenin expression were related with PFS (p < 0.05) (Table 2).

Discussion

Currently, tumor is considered as a disorder at the level of stem cells.8,9 Wnt/β-catenin pathway regulates stem cell proliferation and differentiation, and results in tumorigenesis if activated abnormally. Wnt protein is a signaling molecule while β-catenin is a key component in the pathway, and the accumulation of β-catenin in cytoplasm is critical to the signaling transduction. Wnt signal is absent in normal mature cells and β-catenin is easily to be degraded in cytoplasm. When Wnt signal is aberrantly activated, β-catenin is
accumulated in cytoplasm and translocated to nuclei, which induces transcription of Wnt pathway target genes (such as c-myc and cyclin D1) and finally accelerates cell cycle, facilitates cell proliferation and migration.\(^{10,11}\)

In our study, the high expression rate of β-catenin was 57.7% in NPC samples, and the high expression rate was significantly increased in the cases of advanced clinical stage, indicating that β-catenin expression was associated with tumor load and malignant biological behaviors. The relapse, metastasis and progression rates were also higher in the patients with high expression of cytoplasmic β-catenin than in those with low expression, probably due to cytoplasmic accumulation and nuclear translocation of β-catenin which activates transcription of multiple genes (including EMC and metalloproteinases), promotes tumor invasion and metastasis.\(^{12}\)

Multivariate analysis revealed that high expression of cytoplasmic β-catenin was an independent prognostic factor for post-treatment relapse or metastasis of NPC, suggesting that cytoplasmic β-catenin may be more effective in prognosis prediction and more accurately reflect tumor biological properties than clinical stage. It has been reported that the high level of β-catenin in cytoplasm is associated with bad prognosis in breast cancer,\(^{6}\) colorectal cancer,\(^{7}\) and prostate cancer,\(^{13}\) which was rarely reported in NPC. While our results indicate that cytoplasmic β-catenin was a potential and effective molecular prognostic factor of NPC, which is helpful for treatment decision when considered in combination with clinical stage.

Wnt-1, a member of Wnt protein family, may play an important role in tumorigenesis. However, its correlation to the prognosis of tumors, except for prostate cancer, has seldom reported. Chen et al.\(^{13}\) reported that prostate cancer patients with high Wnt-1 expression were likely to develop distant metastasis, indicating that Wnt-1 is a molecular marker of poor prognosis for prostate cancer. In our study, the high expression rate of Wnt-1 was 61.3% in NPC, irrelevant to patients’ clinical characteristics. Moreover, Wnt-1 showed no effect on RFS, MFS and PFS of NPC patients, suggesting that Wnt-1 may not be related to the prognosis of NPC. Its role in the carcinogenesis of nasopharyngeal epithelia needs to be investigated.

In the study, Wnt-1 was positively correlated to β-catenin with a low correlation coefficient \((r = 0.387)\), their correlations to the prognosis of NPC were different, indicating that there are other potential signals to up-regulate the expression of cytoplasmic β-catenin besides Wnt-1.

Other members of Wnt protein family, such as Wnt-3a, Wnt-8a, Wnt-8b, and so on,\(^{14}\) can also stabilize cytoplasmic β-catenin level to mediate downstream events. Moreover, the activation of PTEN, EGFR pathway,\(^{15}\) and EB virus-mediated PI3/Akt pathway,\(^{16-18}\) or abnormal degradation mechanism of β-catenin can also elevate the concentration of cytoplasmic β-catenin. Therefore, cytoplasmic β-catenin is regulated by multiple factors. Its expression and clinical significance are not necessarily consistent with those of Wnt-1.

Currently, TNM staging is still the main means to predict the prognosis and direct the treatment of NPC, and can be helpful for diagnosis and treatment decision when take molecular prognostic markers into account. According to our results, the high expression rates of Wnt-1 and β-catenin are relatively high in NPC, indicating that Wnt/β-catenin signal transduction pathway may be activated abnormally in some NPC tissues; β-catenin is related to relapse and metastasis, indicating that Wnt protein may play an important role in the development and progression of NPC via β-catenin; β-catenin may be a prognostic marker of NPC.

### Table 2 Multivariate Cox regression analysis for progression-free survival

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>SE</th>
<th>Wald</th>
<th>df</th>
<th>Sig.</th>
<th>Exp (B)</th>
<th>95% CI for Exp (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>1.100</td>
<td>0.539</td>
<td>4.159</td>
<td>1</td>
<td>0.041</td>
<td>3.004</td>
<td>1.044–8.647</td>
</tr>
<tr>
<td>Age</td>
<td>0.374</td>
<td>0.341</td>
<td>1.203</td>
<td>1</td>
<td>0.273</td>
<td>1.454</td>
<td>0.745–2.838</td>
</tr>
<tr>
<td>T stage</td>
<td>0.447</td>
<td>0.575</td>
<td>0.604</td>
<td>1</td>
<td>0.437</td>
<td>1.563</td>
<td>0.507–4.819</td>
</tr>
<tr>
<td>N stage</td>
<td>0.097</td>
<td>0.427</td>
<td>0.052</td>
<td>1</td>
<td>0.820</td>
<td>1.102</td>
<td>0.478–2.543</td>
</tr>
<tr>
<td>‘92 stage</td>
<td>0.328</td>
<td>0.763</td>
<td>0.185</td>
<td>1</td>
<td>0.667</td>
<td>1.388</td>
<td>0.311–6.187</td>
</tr>
<tr>
<td>β-catenin</td>
<td>1.213</td>
<td>0.406</td>
<td>8.950</td>
<td>1</td>
<td>0.003</td>
<td>3.365</td>
<td>1.520–7.450</td>
</tr>
</tbody>
</table>

### References


