Review

Association between class III β-tubulin expression and response to paclitaxel/vinorebine-based chemotherapy for non-small cell lung cancer: A meta-analysis

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A B S T R A C T

Background: It has been proposed that the level of class III β-tubulin gene expression can be used to predict clinical sensitivity to paclitaxel/vinorebine-based chemotherapy in non-small cell lung cancer (NSCLC) patients. However, whereas there are published reports supporting this association, there are also reports of studies that failed to find such an association. We conducted a meta-analysis of all relevant published data to provide a combined statistical assessment of the proposed association of expression variations of class III β-tubulin with objective response and median survival in patients with NSCLC treated with paclitaxel/vinorebine-based chemotherapy.

Methods: We conducted the meta-analysis using data from ten studies, each of which evaluated the correlation between class III β-tubulin expression levels and objective response in patients treated with paclitaxel/vinorebine-based chemotherapy for NSCLC patients. All eligible studies were searched by MEDLINE, EMBASE and CNKI databases. Overall odds ratios (ORs) of the objective response were calculated using the method of Mantel–Haenszel. The differences in objective responses between Caucasian and Asian patients treated with paclitaxel/vinorebine-based chemotherapy were compared. We also compared outcomes for patients treated with paclitaxel to those treated with vinorebine.

Results: There were a total of 552 patients in the ten studies that met our criteria for evaluation. High/positive expression of class III β-tubulin was found in 279 patients (50.5%), and low/negative expression for this gene was found in 273 (49.5%) patients. The objective response rate for paclitaxel/vinorebine-based chemotherapy was significantly higher in patients with low/negative class III β-tubulin expression (OR = 0.28; 95% CI, 0.20–0.41; P < 0.00001). Median survival time was longer for patients with low/negative expression of class III β-tubulin compared with patients with high/positive expression (MR = 1.40; CI, 0.89–0.90; P < 0.00001). There was no significant difference in therapy between Caucasian and Asian patients treated with paclitaxel/vinorebine-based chemotherapy (Chisquared = 0.02, P = 0.88). In our analysis, NSCLC patients treated with paclitaxel had more favorable clinical outcomes than those treated with vinorebine (Chisquared = 3.69, P < 0.05). Conclusions: By combining data from ten different studies, we found a correlation between low TUBB3 gene expression and favorable clinical outcome to anti-tubulin therapy. The correlation for the combined data was significantly stronger than it was for any of the individual studies. This result supports the usefulness of class III β-tubulin mRNA level as a biomarker for sensitivity to paclitaxel/vinorebine-based chemotherapy in NSCLC patients.

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1. Introduction

Lung cancer continues to be the leading cause of cancer death for both men and women worldwide. Non-small cell lung cancer (NSCLC) accounts for approximately 85% in lung cancer cases, and more than half of patients with NSCLC have developed metastasis at the time of diagnosis [1]. Approximately 50% of NSCLC patients receive chemotherapy as part of their treatment [2]. Agents that alter tubulin dynamics, such as paclitaxel and vinorebine, are commonly used in NSCLC treatment, either individually or combined with platinum drugs such as carboplatin or cisplatin. Paclitaxel/vinorebine-based chemotherapy has been shown to increase overall survival time for advanced or inoperable NSCLC [3]. For the patients with early stage disease, surgery followed by a paclitaxel/vinorebine-based chemotherapy is the most effective

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treatment available [4,5]. Several clinical studies have reported that NSCLC patients with high class III β-tubulin expression were more resistant to paclitaxel/vinoreline-based chemotherapy regimens than patients with low levels of expression [6].

Anti-tubulin agents such as paclitaxel and vinoreline inhibit microtubule dynamics, leading to the growth arrest of tumor cells and subsequently cell death [7]. The efficacy of anti-tubulin treatment can be limited by the development of resistance to these agents by cancer cells. The mechanisms controlling resistance to tubulin-binding agents are complex, involving increased expression of multidrug-resistance (MDR) proteins such as the P-gp, changes in tubulin structure, and alterations in the levels of key proteins that control cell survival [8–10]. Another reported source of resistance to these drugs is elevated expression of class III β-tubulin in the cancer tissue. The studies evaluating class III β-tubulin expression variation associated with paclitaxel-based or vinoreline-based chemotherapy have yielded inconsistent results. For several studies, high expression of class III β-tubulin was correlated with an adverse response rate for patients with NSCLC treated with paclitaxel-based or vinoreline-based chemotherapy [11–17]. For other studies, the correlation between tubulin gene expression and clinical response was more ambiguous [18]. Therefore, class III β-tubulin might be a promising prognostic marker for NSCLC treated with paclitaxel-based or vinoreline-based chemotherapy.

However, there has not been a systematic assessment of the literature regarding the association of resistance to paclitaxel/vinoreline-based chemotherapy with high class III β-tubulin expression. Also, most of the individual studies had a small number of patients. Furthermore, most of the studies that investigated the association between paclitaxel-based or vinoreline-based chemotherapy and class III β-tubulin expression involved either one or the other drug, but not both. Therefore, it is still unknown whether there is a significant difference between paclitaxel-based and vinoreline-based chemotherapy in treatment of patients with NSCLC.

We performed a meta-analysis to provide a systematic assessment of whether class III β-tubulin expression is associated with objective response and median survival time in patients with NSCLC based on treatment with paclitaxel/vinoreline-based chemotherapy, and compared difference of objective response between paclitaxel-based and vinoreline-based chemotherapy in treatment for NSCLC.

2. Methods

2.1. Search strategy

We did systematic computerized searches of several databases, including MEDLINE, EMBASE, CNKI, using the keywords “Class III tubulin/tubulin”, “Cancer” and “Chemotherapy”. No language restrictions were applied. The studies were independently evaluated by two reviewers (HLZ, LR), and reached consensus on all items. The following information was recorded from each papers: first author, year, journal, number of patients, number of patients with high/positive class III β-tubulin, number of patients with low/negative class III β-tubulin, age, gender, stage of disease, treatment outcomes (i.e. CR, PR, CR + PR, PD + SD), objective response rate, chemotherapy regimens.

2.2. Eligibility criteria

The studies included in the meta-analysis met the following criteria: (i) utilized paclitaxel-based or vinoreline-based chemotherapy regimens to treat patients with pathologically proven NSCLC; (ii) measured class III β-tubulin gene expression using real-time reverse transcriptase PCR (RT-PCR), immunohistochemistry (IHC), or Western blot (WB); and (iii) provided data for objective response rate and median survival/overall survival based on class III β-tubulin expression variation. Abstracts and papers reported only at academic meetings were excluded for this meta-analysis.

2.3. Assessment of study quality

Two investigators (HLZ, LR) independently assessed the quality of each study using the Newcastle–Ottawa Quality Assessment Scale. Discrepancies were resolved by consensus. The Newcastle–Ottawa Quality Assessment Scale involves assessing three categories – patient selection, study comparability and outcome – based on eight items. Stars awarded to high-quality elements are used to compare study quality in a qualitative manner. Four items in the selection category, two items in the comparability and three items in the outcome category; a maximum of two stars can be given for comparability; a study can be awarded one star for each item in these categories. The scoring system was recommended by the Cochrane Non-randomized Studies Methods Working Group [22,23].

2.4. Statistical analysis

We estimated the odds ratio (OR) for objective response vs. no response to treatment with paclitaxel/vinoreline-based chemotherapy. Objective response is defined as either complete response (CR) or partial response (PR) to treatment using the WHO criteria [24]. Pooled odds ratios and 95% confidence intervals (CIs) were calculated using Mantel–Haenszel method with fixed-effect models [25]. P-value <0.05 was considered as significant for all analysis. Funnel plot analysis was used to estimate publication bias by assessing the relationship between the treatment effects and standard error of the estimate (S.E. log OR). All analyses were performed using software from Stata Corporation (Texas) and Cochrane Collaboration v5.0 (England).

3. Results

3.1. Selection of studies

We identified 326 potentially relevant articles from our initial literature search. We excluded 213 studies after review of the title, because they did not meet the criteria of inclusion. A total of 113 studies were included for abstract review after the first exclusion, and 31 studies were extracted for full text review after careful

| Table 1 | Quality assessment of the studies based on Newcastle–Ottawa Scale (NOS). |
|---------|-------------------------|-----------------|-----------------|-----------------|
| Studies (year) | Selection (stars) | Comparability (stars) | Outcome (stars) |
| Azuma (2009) | 4 | 2 | 3 |
| Rosell (2003) | 3 | 2 | 3 |
| Seve (2005–04) | 4 | 2 | 3 |
| Seve (2005–11) | 4 | 2 | 3 |
| Ukuda (2008) | 3 | 2 | 3 |
| Yang (2008) | 4 | 2 | 3 |
| Yang (2009) | 3 | 2 | 3 |
| Pu (2009) | 3 | 2 | 3 |
| Dumontet (2005) | 3 | 2 | 3 |
| Xiao (2009) | 4 | 1 | 3 |

The Newcastle–Ottawa Quality Assessment Scale comprises three categories: selection, comparability and outcome. The scale uses stars to quantitatively compare the quality of the studies. The maximum number of stars awarded for high-quality elements in each category are four for selection, two for comparability and three for outcome.
abstract review. We excluded 21 studies due to lack of sufficient information or due to lack of clinical data (for example, in vitro chemosensitivity assessments). After completing the selection process, data from a total of ten studies involving 552 patients (Fig. 1) was pooled. The Newcastle–Ottawa Scale was used to conduct the quality assessments for the ten studies. This scale has been adopted in other non-randomized studies [26,27]. Studies which met five or more of the eight criteria were given higher quality scores. A summary of the studies included in this review which scored highly is shown in Table 1. Characteristics of the ten eligible studies are presented in Table 2. Bias assessment was evaluated by funnel plot analysis, shown in Fig. 2.

Fig. 2. Forest plot of comparison for objective response rate between the class III β-tubulin low/negative expression and class III β-tubulin high/positive expression (n = 552), pooled data from these ten studies indicated an overall objective response rate is 22.6% and 49.8% for class III β-tubulin low/negative expression (n = 273) and class III β-tubulin high/positive expression (n = 279). Objective response rate was significantly in favor of class III β-tubulin low/negative expression (OR = 0.28; 95% CI, 0.20–0.41; P < 0.00001).
3.2. Objective response

All of the ten studies reported data on objective response. The fixed-effect model was applied to perform the meta-analysis based on the fact that no heterogeneity was found among studies ($\chi^2 = 9.63, I^2 = 7\%$) [28]. The overall objective response rate was 22.6% for the patient population with high/positive class III $\beta$-tubulin expression ($n = 279$), and 49.5% for the patient population with low/negative class III $\beta$-tubulin expression ($n = 273$). These results indicate a statistically significant favorable clinical outcome for patients with low/negative expression class III $\beta$-tubulin. The pooled odds ratio from the ten studies was 0.28 (OR = 0.28; 95% CI, 0.20–0.41; $P < 0.00001$; see Fig. 3).

Subgroup analysis was conducted based on chemotherapy regimen. Eight studies described clinical results with patients treated with paclitaxel-based chemotherapy, and two clinical studies described results of patients treated with vinorelbine-based chemotherapy. For the paclitaxel-based regimen, the overall objective response rate for patients with low/negative class III $\beta$-tubulin expression was significantly higher than that for patients with high/positive class III $\beta$-tubulin expression (OR = 0.23; 95% CI, 0.15–0.36; $P < 0.00001$). For patients treated with the vinorelbine-based regimen, the patients with low/negative class III $\beta$-tubulin expression enjoyed a significantly better objective response rate (OR = 0.49; 95% CI, 0.26–0.90; $P = 0.02$). There existed substantial heterogeneity between two treatment subgroups ($I^2 = 72.9\%$). The objective response rate difference did reach statistical significance between patients treated with vinorelbine-based chemotherapy and those treated with paclitaxel-based chemotherapy ($\chi^2 = 3.69, P = 0.05$). Interestingly, patients treated with the paclitaxel-based chemotherapy regimen exhibited a better objective response (see Fig. 4).

Six studies were from Asiatic populations and four studies were from Caucasian populations. In the Caucasian subgroup, the subpopulation with low/negative class III $\beta$-tubulin expression had significant response rate (OR = 0.27; 95% CI, 0.14–0.53; $P = 0.0001$). Significant association was also shown for class III $\beta$-tubulin low/negative expression with response rate in Asiatic population subgroup (OR = 0.29; 95% CI, 0.18–0.45; $P < 0.00001$), with no heterogeneity found for both of them. Subgroup analysis indicated that there was no significant difference between Asiatic population and Caucasian population ($\chi^2 = 0.02; 95\% CI, 0.31–0.60; P = 0.88$) (see Fig. 5).

3.3. Median survival time

Seven of the ten studies selected for analysis provided median survival data as shown in Table 3. Overall median survival time was 56 weeks for the patients with high/positive class III $\beta$-tubulin expression treated with paclitaxel/vinorelbine-based chemotherapy, whereas, overall median survival time was 78 weeks for the patients with low/negative class III $\beta$-tubulin expression. Pooled median ratios were calculated by weighting the mean of median survival time from individual studies. Low/negative class III $\beta$-tubulin expression significantly prolonged median survival time for NSCLC patients treated with paclitaxel/vinorelbine-based chemotherapy (MR = 1.40; 95% CI, 0.89–0.90; $P < 0.0001$).

4. Discussion

In this meta-analysis, we analyzed pooled data from ten clinical studies, each of which measured the level of class III $\beta$-tubulin gene expression in NSCLC patients treated with anti-tubulin agents [6,12–17,19–21]. Our goal was to test the hypothesis that low class III $\beta$-tubulin expression is associated with better objective response rate and longer median survival time. We found that the objective response rate of patients with low/negative class III $\beta$-tubulin expression treated with paclitaxel/vinorelbine-based chemotherapy was significantly higher than that in patients with high/positive class III $\beta$-tubulin expression. The meta-analysis presented here supports the utility of testing lung cancer tissue for TUBB3 gene expression to help guide selection of clinical chemotherapy regimens involving anti-tubulin agents.

This meta-analysis indicated that class III $\beta$-tubulin low/negative expression had a better prognosis than class III $\beta$-tubulin high/positive expression. The meta-analysis was just
performed for the patients with NSCLC. However, individual research reported in breast and gastric cancer patients treated with paclitaxel/vinorebine-based chemotherapy was concordant with this meta-analysis result [29–31]. Therefore, this result may be applicable for other cancers such as breast and gastric cancers, though we did not perform a systematic analysis for breast and gastric cancers.

All of the ten studies indicated a higher objective response for class III β-tubulin low/negative expression than for class III β-tubulin high/positive expression, and there was no observed heterogeneity across the ten studies ($I^2 = 7\%$). We further investigated the heterogeneity by performing subgroup analysis. The heterogeneity was moderate at 44% in Caucasian subgroup, which might be due to difference of chemotherapy regimen.

**Fig. 4.** Forest plot of subgroup analysis comparison: in the subgroup of the patients received with paclitaxel-based chemotherapy, pooled objective response rate of class III β-tubulin low/negative expression was significantly higher than that of class III β-tubulin high/positive expression (OR = 0.23; 95% CI, 0.15–0.36; $P < 0.00001$). In vinorebine-based chemotherapy subgroup, patients with class III β-tubulin low/negative expression predicted better objective response rate (OR = 0.27; 95% CI, 0.41–0.53; $P = 0.0001$). There existed substantial heterogeneity between two treatment subgroup ($Chi^2 = 3.69$, $P = 0.05$, $I^2 = 72.9\%$).

**Fig. 5.** Forest plot of subgroup analysis comparison: in Caucasian subgroup, class III β-tubulin low/negative expression was significantly associated with response rate (OR = 0.27; 95% CI, 0.14–0.53; $P = 0.0001$). In Asiatic population subgroup, response rate of class III β-tubulin low/negative expression was significantly higher than that of class III β-tubulin high/positive expression (OR = 0.29; 95% CI, 0.18–0.45; $P = 0.00001$), there was no heterogeneity found between two subgroups.
Subgroup analysis based on the use of either vinorelbine or paclitaxel chemotherapeutic agents was conducted to evaluate differences in their performance. In eight studies patients received a paclitaxel-based chemotherapy regimen, and two studies treated with vinorelbine-based chemotherapy regimen. Paclitaxel-based chemotherapy regimen (P < 0.0001) performed a higher objective response compared with vinorelbine-based chemotherapy regimen (P = 0.03). The knowledge gained from this subgroup analysis implies that treatment with paclitaxel-based chemotherapy regimen may be more beneficial for patient with NSCLC, compared with vinorelbine-based chemotherapy regimen. However, evaluation of a larger data set containing more prospective studies and a larger sample size would provide greater confidence in this apparent difference in efficacy between the two anti-cancer agents.

Publication bias is a possible limitation for this meta-analysis because negative results or results opposed to mainstream theories are less likely to be published than papers reporting positive results. However, we did not find that publication bias significantly influences our result of the meta-analysis.

In conclusion, this meta-analysis provided evidence that class III β-tubulin low/negative expression is associated with higher response rate and has longer survival time for the cancer patient treated with paclitaxel/vinorelbine-based chemotherapy. Class III β-tubulin could be a very useful biomarker for prognosis and sensitivity to paclitaxel/vinorelbine-based chemotherapy for the patients with NSCLC.

Conflict of interest statement

None of the authors has any conflict of interest.

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