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Research paper

High expression of topoisomerase-II predicts favorable clinical outcomes in patients with relapsed small cell lung cancers receiving amrubicin

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ABSTRACT

Objectives: Amrubicin monotherapy is a treatment option for patients with relapsed small cell lung cancers (SCLCs). Topoisomerase-II (Topo-II) – a target of amrubicin – has been reported as a predictive or prognostic marker for chemosensitivity or outcomes in patients with various malignancies. Here, we investigated the prognostic role of Topo-II expression in patients with relapsed SCLCs who underwent amrubicin monotherapy.
Materials and methods: Eighty-three patients with relapsed SCLCs who received amrubicin monotherapy between 2004 and 2015, after progression beyond first-line chemotherapy, were enrolled in the study. We retrospectively collected clinical data from their medical records, and evaluated the expression levels of Topo-II, by immunohistochemical staining of archival tumor specimens obtained through surgical resections or biopsies.
Results: Most of the enrolled patients were elderly men (89%), with a median age of 70 years (range, 49–83); 16% of these patients showed Topo-II overexpression. Compared to patients with sensitive relapses, those with refractory relapses showed significantly higher Topo-II expression levels ($P = 0.03$). The overall response rates in patients with high and low Topo-II expression were 38.5% and 25.7%, respectively ($P = 0.34$). Multivariate analysis confirmed that patients with a higher Topo-II expression level had significantly longer progression-free survival (hazard ratio (HR), 0.39; $P < 0.01$) and overall survival (HR, 0.48; $P = 0.04$), compared to patients with a lower Topo-II expression level.
Conclusion: Our study identified Topo-II expression as a significant biomarker for the prediction of favorable outcomes in patients with relapsed SCLCs who underwent treatment with amrubicin, a Topo-II inhibitor. Thus, Topo-II expression may be a promising predictor of the efficacy of amrubicin.

1. Introduction

Small cell lung cancers (SCLCs) are distinct neuroendocrine tumors with aggressive features, and account for 13% of all newly diagnosed cases of lung cancer [1]. Despite showing high response rates to initial combination chemotherapy [2,3], most patients with SCLCs experience either recurrences or disease progression. Thus, most patients with relapsed SCLCs need effective salvage chemotherapy. However, standard chemotherapy for this purpose has not yet been established despite extensive efforts to develop new strategies for relapsed SCLCs.

In the United States, topotecan, a specific DNA topoisomerase-I inhibitor, is the only agent approved for second-line therapy in patients with relapsed SCLCs, from among the agents recommended in the National Comprehensive Cancer Network (NCCN) guidelines [4] (based on the results of previous randomized phase III trials [5,6]). However, the results of a Japanese prospective study of patients treated with topotecan were disappointing, especially those pertaining to refractory relapses, demonstrating overall response rates (ORRs) of 0%, a median progression-free survival (PFS) of 1.5 months, and a median overall survival (OS) of 5.4 months [7].

Amrubicin, a fully synthetic 9-aminoanthracycline derivative, is converted to the active metabolite amrubicinol in the body via the reduction of a ketone motif at its 13th position; this serves as a DNA topoisomerase-II (Topo-II) inhibitor, and not mainly as a DNA intercalator [8]. In Japan, amrubicin was approved for use in 2002, and is one of the treatment options available for patients with relapsed SCLCs.

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Several phase II studies have shown promising efficacy of amrubicin in patients, with ORRs of 21–67%, median PFS of 3.2–5.4 months, and median OS of 6.0–14.4 months [7,9–12]. Although the largest randomized phase III trial conducted could not confirm the superiority of amrubicin therapy over that of topotecan therapy in terms of patient survivals, subset analysis indicated that SCLC patients with refractory relapses could benefit greatly from undergoing amrubicin therapy [13]. This has encouraged further investigations into the clinical characteristics or biological features that may result in higher anti-tumor effects of amrubicin therapy. To date, to the best of our knowledge, no studies have elucidated any potential biomarkers that may predict tumor responses or clinical outcomes in patients with SCLC treated with amrubicin, except in case of a polymorphism of NAD(P)H quinone oxidoreductase 1 [14]; however, the predictive value of this biomarker has not been validated in prospective trials.

Topo-II, a target of amrubicin, has been evaluated as a potential biomarker in various malignancies, including breast [15,16], ovarian [17], and lung cancers [18,19]. A large-scale prospective pooled analysis reported that Topo-II expression was a significant biomarker in predicting the benefits of adjuvant anthracycline chemotherapy in breast cancer [16]. However, its role as a biomarker remains unclear in patients with relapsed SCLCs who receive amrubicin therapy. Therefore, our present study examines whether the expression levels of Topo-II, determined by immunohistochemistry, can be correlated with chemosensitivity or clinical outcomes in patients with relapsed SCLCs who received amrubicin monotherapy.

2. Material and methods

2.1. Patients

We screened 116 patients with either SCLC or large cell neuroendocrine carcinoma (LCNEC) treated at the Shibukawa Medical Center or the Gunma University Hospital between July 2004 and July 2015. All patients experienced relapse after first-line chemotherapy, and subsequently received amrubicin monotherapy. Thirty-three patients were excluded due to non-availability of tumor specimens (n = 21), inaccurate diagnoses (n = 2), or receipt of amrubicin as first-line therapy (n = 10). A total of 83 patients were eventually enrolled in the present study.

2.2. Data collection

We retrospectively collected data on patient characteristics such as response to chemotherapy, pathological findings, and survival, from patient medical records. The clinical stage at diagnosis was classified into limited disease (LD) or extensive disease (ED) [20]. The histopathological types were assessed according to the 2004 World Health Organization histological classification [21]. Types of relapse were classified as sensitive or refractory relapses, according to the length of the treatment-failure interval (TFI). We defined TFI as the period from the date of completion of first-line therapy to the date of recurrence. As defined in most clinical trials, relapses with TFI ≥ 90 days were defined as sensitive relapses; relapses with TFI < 90 days were defined as refractory relapses. The tumor responses were assessed according to the Response Evaluation Criteria in Solid Tumors version 1.1 [22]. PFS was defined as the time interval between the date of amrubicin treatment initiation and the date of disease progression or of death due to any cause. Similarly, OS was calculated as the time interval between the date of amrubicin treatment initiation and the date of death or the last follow-up consultation. This study was conducted in compliance with the Declaration of Helsinki. The study protocol was approved by the Institutional Review Boards of the Gunma University Hospital (ref. 1287) and the National Hospital Organization Shibukawa Medical Center (ref. 15-03-05).

2.3. Immunohistochemical analysis

Tumor samples were obtained via surgical resections or biopsies obtained prior to first-line chemotherapy; 10 samples (12%) were obtained via surgical resections, and 69 (83%) via biopsies. Information about the collection procedure was not available for 4 samples (5%). The procedure used for immunohistochemical staining has been described previously [23]. An anti-Topo-II rabbit polyclonal antibody (ab180393, Abcam, Tokyo, Japan, 1:100 dilution) was used in this study. Cells were deemed positive for Topo-II if positive staining was present in the nuclei. The proportion of Topo-II-positive cells was assessed by using a semi-quantitative scoring method, wherein samples were assigned a score based on the percentage of positive cells: Score 1, < 10% positive cells; 2, 10% to < 25%; 3, 25 to < 50% positive cells; 4, 50 to < 75%, and 5, ≥75% positive cells [24,25]. We compared tumor responses and survival data between the groups that showed high (Topo-II-high group) and low (Topo-II-low group) Topo-II expression, with various cut-off scores for Topo-II expression. In the present study, expression scores between 1 and 4 signified low Topo-II expression, and a score of 5 signified high Topo-II expression. Given that most samples were biopsy specimens, only the presence, but not the intensity, of the staining was used for analysis. Sections were examined under light microscopy by at least two investigators in a blinded fashion. In case of discrepancies, both investigators simultaneously evaluated the slides until a consensus was reached.

2.4. Statistical analysis

Statistical significance was set at $P < 0.05$. The association between immunohistochemical staining and the clinicopathological factors was examined using the Fisher's exact test. The difference in mean Topo-II scores between the two groups was analyzed by the nonparametric Mann-Whitney test. The Kaplan-Meier method was used to estimate survivals, and the survival difference between groups was analyzed by the log-rank test. Multivariate analyses were performed using the Cox proportional hazards model to identify independent prognostic factors. Statistical analysis was performed using GraphPad Prism 6 software (Graph Pad Software, San Diego, CA, USA) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) for Windows.

3. Results

3.1. Patient characteristics according to Topo-II expression

The characteristics of the patients included in this study are summarized in Table 1A. Most eligible patients were elderly men (89%), with a median age of 70 years (range, 49–83). Most tumor specimens showed histology characteristic of SCLC (92%). Sixty-four patients (77%) had favorable performance status (PS) scores of 0 or 1, while 19 patients (23%) had unfavorable PS scores of 2, 3, or 4. Sensitive relapses occurred in 24 patients (29%), and refractory relapses in 59 (71%). Amrubicin was administered as second-line therapy in 61 patients (74%), and administered as third-line or more in 22 patients (26%). After progression beyond amrubicin monotherapy, 49 patients (59%) received subsequent chemotherapy, whereas 34 (41%) received no further chemotherapy.

The clinicopathological features of patients according to Topo-II expression are summarized in Table 1B. The Topo-II-high group had a significantly lower proportion of patients treated with irinotecan-based chemotherapy, compared to the Topo-II-low group ($P = 0.03$). More refractory relapses and lower incidence of subsequent chemotherapy after amrubicin monotherapy were seen in the Topo-II-high group, although these differences were not statistically significant. The patients in the Topo-II-high group tended to receive amrubicin as second-line therapy rather than as third-line or more.