ERC/Mesothelin as a Marker for Chemotherapeutic Response in Patients with Mesothelioma

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Abstract. *Background: It has been recently reported that soluble mesothelin-related protein (SMRP), serum mesothelin, and osteopontin (OPN) are considered as relevant biomarkers for the diagnosis of mesothelioma. The aim of this study was to investigate whether serum N-ERC/mesothelin, an NH_3-terminal fragment of mesothelin, and plasma OPN reflect chemotherapeutic effect in patients with mesothelioma. Materials and Methods: Serum N-ERC/mesothelin and plasma osteopontin were determined with a sandwich enzyme-linked immunosorbent assay (ELISA) system. Results: The average N-ERC ratio, determined by dividing the N-ERC levels following chemotherapy by those prior to chemotherapy, in the partial response (PR) group was significantly lower than that of the stable disease (SD)/progressive disease (PD) group. In contrast, the average OPN ratio, determined by dividing the OPN levels following chemotherapy by those prior to chemotherapy, in the PR group was not statistically different from that of the SD/PD group. Conclusion: N-ERC/mesothelin is considered as relevant in monitoring chemotherapeutic response in patients with mesothelioma.*

Mesothelioma is an extremely invasive tumor and has been demonstrated to be resistant to all conventional therapeutic regimens. For patients who were unable to attain curative surgical results, the median survival duration has been reported as 6 months (1). Results of chemotherapeutic treatment have been disappointing, although recent studies have described the usefulness of new anti-metabolic agents in combination with platinum (2). Mesothelioma is extremely difficult to assess for response, with the tumor growing and surrounding the hemithorax and along the interlobar fissure. Although tumor measurement in mesothelioma provides many challenges for clinical investigators, conventional evaluation of chemotherapeutic response has been based on computed tomography (CT) measurements (3-7). However, accurate evaluation is sometimes difficult to attain. Therefore, the development of an easier and cost efficient tool for determining chemotherapeutic response in patients with mesothelioma is required. Serum biomarkers may be an ideal tool for this purpose. Various tumor markers have been used for the diagnosis and monitoring of chemotherapeutic response in other malignancies. For instance, prostate-specific antigen (PSA) and CA125 are routinely used in the clinical setting to monitor therapeutic effects in prostate cancer and ovarian cancer, respectively (8, 9). However, there are only a few markers showing both high sensitivity and specificity

It has been recently reported that soluble mesothelin-related protein (SMRP), serum mesothelin, and osteopontin (OPN) are useful biomarkers for the diagnosis of mesothelioma (10-13). We have previously identified the expressed in renal carcinoma (*ERC*) gene, which is highly expressed in renal carcinomas in the Eker rat (14, 15). We have also demonstrated that ERC is a homolog of the human *mesothelin* gene, which is the causative gene for mesothelioma. The human mesothelin gene product is cleaved with furin-like protease to a 31-kDa *N*-terminal fragment (N-ERC/mesothelin) that is secreted into the blood

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(16). We have developed an enzyme-linked immunosorbent assay (ELISA) system that detects N-ERC/mesothelin (17-19) and have recently shown that N-ERC/mesothelin is useful for the early diagnosis of mesothelioma with both high sensitivity and high specificity.

However, no studies have addressed the clinical importance of these markers in monitoring chemotherapeutic response. The aim of this study was to investigate whether serum N-ERC/mesothelin and plasma OPN correlate to the chemotherapeutic effect, and to compare their efficacy with regards to the prediction of chemotherapeutic response of patients with mesothelioma.

Materials and Methods

Patients. The study was approved by the Institutional Review Board of Juntendo University, School of Medicine. Written informed consent was obtained from all the patients. Blood was obtained prior to and following a minimum of one course of chemotherapy from 14 patients with pleural mesothelioma between June 2005 and May 2008 at our hospital. The patient characteristics are shown in Table I. Briefly, the chemotherapy regimens included the combination of pemetrexed and cisplatin, cisplatin and gemcitabine and carboplatin and gemcitabine. The chemotherapeutic responses based on modified Response Evaluation Criteria in Solid Tumors (RECIST), were graded as partial response (PR, 21%), stable disease (SD, 35%) and progressive disease (PD, 43%).

Preparation of anti-ERC/mesothelin antibodies. The anti-N-ERC/mesothelin monoclonal antibody (MoAb) clones 7E7 and 16K16 have been previously reported (17, 19). Briefly, N-ERC mesothelin, expressed in *Eschericia coli* as glutathione-*S*-transferase - and histidine-tagged fusion protein, was purified and used as an immunogen. Splenocytes from immunized mice were fused with the myeloma cell line, X63-Ag8.653. Using ELISA, the supernatants of the hybridoma cells were screened by their reactivity to the immunogen and several positive clones were selected with the limiting dilution method. 7E7 and 16K16 were selected for ELISA analysis.

ELISA assays. Serum N-ERC/mesothelin was determined with the sandwich ELISA system using 7E7 and 16K16 mAbs. The details have been previously described (17, 19). Plasma OPN was determined with a human OPN ELISA kit (Immuno-Biological Laboratoreis, Gunma, Japan) according to the manufacturers' instructions.

Statistics. Statistical analysis was performed with the unpaired *t*-test using the Statistical Package for the Social Sciences (SPSS) version 15.0F (SPSS Inc., Chicago, IL, USA). Differences between levels were considered statistically significant at $p<0.05$.

Results

Serum N-ERC/mesothelin level. The N-ERC levels of all the SD/PD patients increased following chemotherapy. In contrast, the N-ERC levels in all except one case among the PR patients decreased following chemotherapy. The average ratio obtained by dividing the N-ERC levels following chemotherapy by those prior to chemotherapy was 1.77±0.65 in the SD/PD group and 0.71±0.69 in the PR group. The average ratio in the PR group was significantly lower than that of the SD/PD group ($p<0.05$) (Figure 1).

Table I. *Characteristics of the patients with pleural mesothelioma.*

Age (years)	
Average (range)	61 (47-78)
Gender	
M / F	9 / 5
Histology	
Epi / Sar / Bi	11 / 2 / 1
Stage	
I / II / III / IV	1 / 3 / 3 / 7
Regimen	
CDDP + Pem / CDDP + GEM / CBDCA + GEM	9 / 2 / 3
Response	
PR / SD / PD	3 / 5 / 6
N-ERC (ng/ml)	
Average (range)	22.25 (1.62~97.5)
OPN (ng/ml)	
Average (range)	1149.29 (366.28~4888.32)

CDDP: cisplatin, Pem: pemetrexed, CBDCA: carboplatin, GEM: gemcitabine; Epi: epithelioid, Sar: sarcomatoid, Bi: biphasic; PR: partial response, SD: stable disease, PD: progressive disease.

Plasma OPN level. The OPN levels of all the PR patients decreased following chemotherapy. In contrast, the OPN levels of the SD/PD patients were variable following chemotherapy. The average ratio obtained by dividing the OPN levels following chemotherapy by those prior to chemotherapy was 1.58±1.02 in the SD/PD group and 0.56±0.39 in the PR group. The average ratio in the PR group was slightly lower than that in the SD/PD group, however, this difference was not statistically significant (Figure 1).

Discussion

In this study, N-ERC/mesothelin in serum was shown to be an ideal biomarker for monitoring chemotherapeutic response and to our best knowledge, there have been no reports with regard to monitoring markers for meosthelioma.

As expected, serum N-ERC/mesothelin was apparently superior to plasma OPN in monitoring chemotherapeutic response in patients with mesothelioma, since the average N-ERC/mesothelin ratio was significantly higher in the PR group than that of the SD/PD group. The superiority of N-ERC/mesothelin to OPN could be explained by the observation that OPN is expressed not only by mesothelial cells, but also by a variety of cells including osteoclasts,

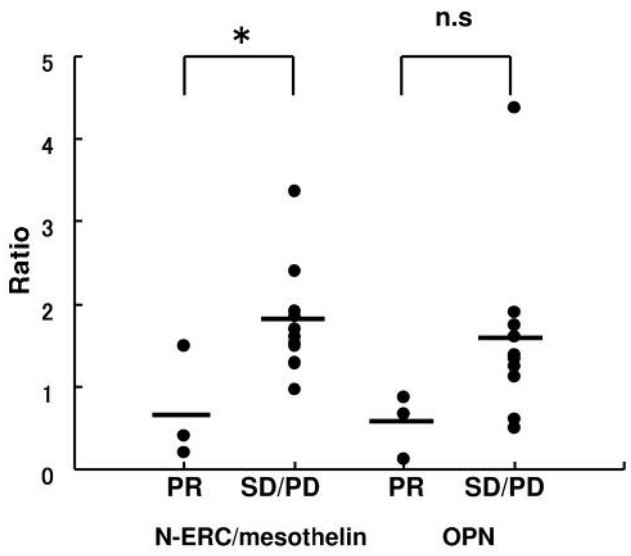

Figure 1. *Comparison of N-ERC/mesothelin ratio and OPN ratio in responding and non-responding patients. N-ERC/mesothelin and OPN ratios were calculated by dividing the serum N-ERC or OPN value following chemotherapy by the value prior to chemotherapy, respectively. *p<0.05 vs. PR.*

activated T-cells and macrophages in normal and various pathological conditions, while mesothelin is expressed by normal mesothelial cells and highly expressed exclusively in mesothelioma and ovarian carcinoma (20-24). These results taken with our previous reports suggested that serum N-ERC/mesothelin is a more reliable marker than plasma OPN not only for diagnosis, but also in monitoring chemotherapeutic response in patients with mesothelioma.

We have recently revealed that serum N-ERC levels were elevated in and more reliable for the diagnosis of epithelioid type mesothelioma than the biphasic and sarcomatoid type mesothelioma. In the present study, one case each of sarcomatoid type and biphasic type mesothelioma were enrolled and, as expected, N-ERC/mesothelin levels prior to chemotherapy in these two patients were relatively low (data not shown). However, interestingly, the levels of N-ERC/mesothelin increased in both patients in parallel with disease progression (data not shown). These results suggested that serum ERC/mesothelin might also be useful for monitoring response to chemotherapy even in patients with sarcomatoid or biphasic type mesothelima. However, since only two patients were involved, the results were not conclusive.

ERC/mesothelion is cleaved into a 40-kDa *C*-terminal fragment (C-ERC/mesothelin) that adheres to the cell surface, as well as the 31-kDa N-ERC/mesothelin that is secreted into the blood (16). The N-ERC/mesothelin may be more sensitive for early diagnosis and reliable for evaluating chemotherapeutic response in patients with mesothelioma in comparison to the C-ERC/mesothelin or SMRP, which are detected by the commercially available MESOMARK kit®. At present, no data are available as to which is a more reliable or valuable marker for monitoring chemotherapeutic response to treatment, and future studies are required.

In summary, our study, although conducted with a small number of patients, revealed that N-ERC/mesothelin is useful in monitoring chemotherapeutic response in patients with mesothelioma. Due to difficulties in assessing and evaluating therapeutic response based on only CT scan measurements, it is essential that a prospective study to investigate and establish the clinical utility of N-ERC/mesothelin using our novel ELISA system is conducted. The use of N-ERC/mesothelin evaluation in combination with CT scan measurements may be useful for improving the accuracy and reliability of evaluating therapeutic response to chemotherapy in patients with mesothelioma.

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