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Short Communication

Sestrin-2 is significantly increased in malignant pleural effusions due to lung cancer and is potentially secreted by pleural mesothelial cells

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ABSTRACT

Objectives: Sestrin-2 (Sesn2) belongs to a family of highly conserved antioxidant proteins that were discovered as p53-inducible proteins and inhibits cell growth and proliferation. Our aim was to assess the levels of Sesn2 in malignant pleural effusions of lung cancer patients compared to benign pleural effusions.

Design and methods: We enrolled 73 patients (55/males and 18/females) diagnosed with pleural effusion (PE). PEs were grouped as 44 malignant pleural effusions (MPEs; lung cancer) and 29 benign (BPE; 7 congestive heart failure, 9 tuberculosis, 13 parapneumonic). Pleural fluid (PF) Sesn2 levels were determined by enzyme-linked immunosorbent assay (ELISA) kit. Standard biochemical PF analysis was also performed and Sesn2 levels were correlated with PF lactate dehydrogenase (LDH), protein, cell counts and age.

Results: Sesn2 was detected in 24/44 patients with MPEs and in 3/29 patients with BPEs ($p = 0.0001$). The mean value (mean ± SEM) of Sesn2 in patients with MPEs was 0.54 ± 0.22 ng/mL while in BPEs it was 0.12 ± 0.04 ng/mL ($p = 0.0004$). In MPEs Sesn2 pleural fluid levels did not correlate with PF LDH and cell counts ($p = 0.89$ and $p = 0.64$ respectively).

Conclusions: Our study shows that Sesn2 is significantly increased in MPEs compared to BPEs. Moreover, the lack of correlation of Sesn2 levels with PF cell counts and PF LDH suggests that it is potentially secreted by pleural mesothelial cells.

1. Introduction

Pleural effusion (PE) is the most common manifestations of pleural involvement in many diseases. A PE formed due to malignancy is termed malignant pleural effusion (MPE) and the most common causes of MPEs are primary lung and breast cancer [1]. In the context of development of a PE many factors pertinent to cancer that alter the permeability of the pleura and its microvasculature come into play, ranging from the protein content in the pleural fluid to stress hormones and growth factors [2–6].

Sesn2 belongs to a family of highly conserved antioxidant proteins transcriptionally regulated by tumor suppressor p53, the most commonly mutated gene in cancers [7–9]. Overexpression of Sesn2 can suppress the target rapamycin (TOR) complex-1 (TORC-1) pathway that is often activated in human cancers while in lung adenocarcinomas Sesn2 expression is down-regulated [10]. Moreover, Sesn2 may have both tumor-suppressive and metastasis promoting activities when counteracting with transforming growth factor beta (TGF-β) [11]. In PEs, it has been shown that components of the oxidant–antioxidant equilibrium are capable of altering pleura permeability as well as to be overexpressed in exudative pleural effusions rendering support to the notion that oxidative stress may is a critical component of the pleural effusion formation [12–14].

The aim of this study was to assess the levels of Sesn2 in patients with PEs so as to show that it is detected in pleural fluid and to compare whether its levels differ between MPEs and benign pleural effusions (BPEs). Moreover, we aimed at correlating Sesn2 levels in pleural fluid

Abbreviations: Sesn2, Sestrin-2; MPE, malignant pleural effusion; BPE, benign pleural effusions; PF, pleural fluid; LDH, lactate dehydrogenase; TORC-1, target rapamycin (TOR) complex-1; TGF-β, transforming growth factor beta; CHF, congestive heart failure; TB, tuberculosis; PPE, parapneumonic pleural effusions; ROC, receiver operator characteristics; AUC, area under the curve; PLR, positive likelihood ratio; NLR, negative likelihood ratio; SOD1, superoxide dismutase-1; Nrf2/Keap1, Nuclear factor erythroid 2 (NF-E2)-related factor 2/Kelch-like ECH-associated protein 1.

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with total cell counts and lactate dehydrogenase (LDH) in order to investigate its route of delivery to pleural fluid.

2. Materials and methods

2.1. Patients

This study included 73 patients, 55 males and 18 females (mean age of 61.45 ± 13.33 years X ± S.D.) that were diagnosed with pleural effusions of different etiologies and who were hospitalized in the Department of Respiratory Medicine of the Faculty of Medicine of the University of Thessaly in Larissa between February 2011 and February 2012 and underwent diagnostic thoracentesis. Pleural effusions were divided to MPEs (44) and BPEs (29). More specifically all the MPEs originated from patients with lung cancer while the BPEs were from 3 different categories [7 congestive heart failure (CHF), 9 tuberculosis (TB) and 13 parapneumonic (PPE)]. The local ethics committee approved the study protocol and all subjects provided written informed consent.

2.2. Sample collection, analysis and diagnostic criteria for pleural effusions

Pleural fluid samples were collected from all patients upon hospital admission. Samples were immediately analyzed by standard commercially available methods for measuring total cell counts, total protein and LDH as described previously [12]. The criteria used for the establishment of diagnosis of pleural effusions by our group have been reported previously [12]. Aliquots of pleural fluid samples were immediately centrifuged at 1500 *g* for 15 min at 4 °C to pellet the cellular elements and the supernatants were stored at −80 °C for Sesn2 evaluation.

2.3. Sesn2 measurements

We measured the levels of Sesn2 in pleural fluid samples using commercially available enzyme-linked immunosorbent assay kit (ELISA) [USCN LIFE]. The lower limit of detection for Sesn2 was 0.061 ng/mL. Sesn2 levels that were detected above the limit of detection in MPEs were correlated with pleural fluid total cell counts and LDH.

2.4. Statistical analysis

The normality of the distribution of data was checked using the D'Agostino & Pearson Omnibus normality test. Comparisons of frequencies were performed with χ^2 test while comparisons of means were performed with Mann–Whitney or Kruskal–Wallis with Dunn post-hoc test. Univariate correlation for skewed (not normally distributed) data correlation was performed with the non-parametric Spearman correlation test. A two-sided *p*-value of <0.05 was considered statistically significant. The above tests were performed with GraphPad Prism version 5.0 for Mac (GraphPad Software, San Diego, Ca USA).

To evaluate the diagnostic performance of Sesn2 in differentiating between MPEs and BPEs, receiver operator characteristics (ROC) analysis was constructed and area under the curve (AUC) was calculated. The optimum cut-off point from the ROC analysis was established by selecting the value that provides the greatest sum of sensitivity and specificity, i.e. the point closest to the upper left corner of the ROC plot. For the optimum cut-off point provided by the ROC analysis, sensitivity, specificity, positive likelihood ratio (PLR), and negative likelihood ratio (NLR) were calculated using standard formulas. For the calculation of the ROC curves and AUCs we used the GraphPad Prism version 5.0 for Mac and for the PLR and NLR we used the MedCalc Free Statistical Calculator for diagnostic test evaluation (https://www.medcalc.org/calc/diagnostic_test.php).

3. Results and discussion

Sesn2 was detected in 24/44 patients with MPEs and in 3/29 patients with BPEs (detection limit, 0.061 ng/mL; $\chi^2 = 14.65$; $p = 0.0001$). This means that Sesn2 levels in pleural fluid of patients suffering from PEs due to etiologies other than lung malignancy are unlikely to be detected. Indeed in the analysis that involved comparisons of the pleural fluid levels of Sesn2 in MPEs versus BPEs showed that Sesn2 levels in MPEs were significantly higher compared to BPEs (0.54 ± 0.22 ng/mL versus 0.12 ± 0.04 ng/mL; $p = 0.0004$) (Fig. 1A). Since no other published studies exist on this area, our results indicate that Sesn2 levels in pleural fluid could be a potentially useful biomarker for the differentiation of malignant and benign PEs.

For this purpose, ROC analysis evaluated the diagnostic performance of pleural fluid Sesn2 in the detection of MPEs. AUC for Sesn2 was

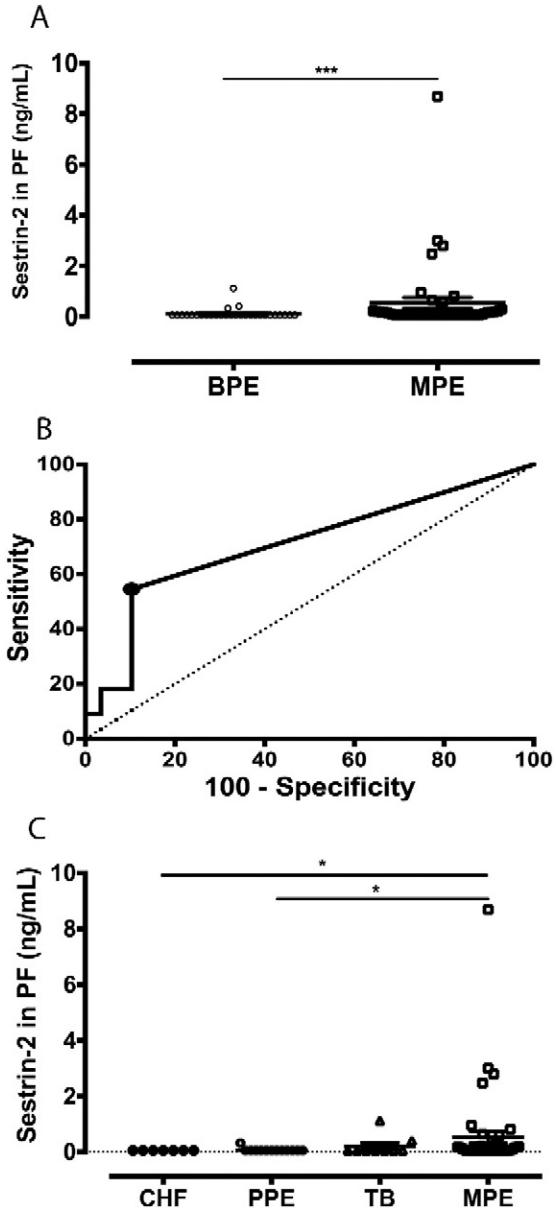

Fig. 1. (A) Comparison of pleural fluid Sesn2 levels in MPEs versus BPEs. MPE levels of Sesn2 in pleural fluid are significantly increased compared to BPEs. $^{***}p < 0.001$. (B) Receiver operator characteristic (ROC) analysis curve of Sesn2 in pleural fluid for the differentiation of BPEs and MPEs. ROC curve of Sesn2 for the differentiation of MPEs vs. BPEs, (optimal cut-off point, >0.065 ng/mL). (C) Comparison of pleural fluid Sesn2 levels in CHF, PPE, TB and MPEs due to lung cancer. MPE levels of Sesn2 in pleural fluid are significantly increased compared to CHF and PPE. $^{*}p < 0.05$.

Table 1
Correlation of Sesn-2 pleural fluid levels with LDH, and total cell counts in lung cancer MPEs.

Spearman correlation coefficient (ρ)	
LDH	Cell counts
ρ = −0.03 [−0.44 to 0.39] p = 0.89	ρ = −0.10 [−0.49 to 0.33] p = 0.64

calculated to be 0.71 (0.59–0.83; 95% CI) and had a value of $p = 0.0027$, indicative of its potential to discriminate MPEs from BPEs (Fig. 1B). A cutoff point of >0.065 ng/mL had low sensitivity 55.54% and high specificity 89.66% for the diagnosis of a MPE. The PLR for detecting a MPE by Sesn2 pleural fluid measurement was 5.27 while the NLR was 0.21. These findings suggest that the application of Sesn2 as a biomarker for the differentiation between MPEs and BPEs is potentially useful especially as a confirmation test rather than a screening test, since its sensitivity is low.

In a sub-analysis that we performed in order to compare the levels of Sesn2 in PEs of different etiologies as shown in Fig. 1C, its levels in MPEs were significantly increased as compared to CHF and PPEs ($p < 0.05$ in both cases) but not as compared to TB. This finding demonstrates that pleural fluid Sesn2 cannot distinguish between all types of infectious PEs but it is adequate to differentiate only between CHF and PPEs.

Subsequently, we decided to investigate whether the levels of Sesn2 that were measured to be above the limit of detection (24/44) correlated with pleural fluid LDH and total cell counts. We performed univariate analysis and according to our results there was no significant correlation of the pleural levels of MPEs Sesn2 and LDH and total cell counts ($p = 0.89$ and $p = 0.64$ respectively; Table 1). The absence of a correlation between pleural fluid LDH, a surrogate marker of cell death, and Sesn2 suggests that Sesn2 is not released into the pleural fluid due to cell death in which case a strong correlation of Sesn2 and LDH would occur. Similarly the lack of correlation between pleural fluid Sesn2 and total cell counts suggests that Sesn2 is not released in the pleural cavity by inflammatory white cells. All of the above support the notion that Sesn2 is potentially secreted by pleural mesothelial cells, something that has not been investigated previously so no published data to support or reject this finding exist.

The physiological basis of our findings requires further investigation, as the role of Sesn2 in pleural pathophysiology has not been studied at all. Sesn2 is pivotal in p53-dependent antioxidant defenses because it reduces the oxidized form of peroxiredoxins and by inhibiting the TORC-1 anabolic pathway [8]. Although pleural mesothelial cells express peroxiredoxins, they have not been investigated in pleural fluids, however peroxiredoxin-1 has been found in high quantities in ascetic fluids from patients with epithelial ovarian cancer [15]. This suggests that a functional target of Sesn2 is probably secreted in serosal fluids, such as pleural fluid, during malignancies and sets the stage for the hypothesis that pleural fluid Sesn2 found in our study possibly occurs for the regeneration of the extracellular anti-oxidant defenses. This hypothesis is further supported by the fact that in MPEs the oxidative stress is increased and that other important anti-oxidant proteins have been detected in the pleural fluid in other studies such as superoxide dismutase-1 (SOD1) and DJ-1 [12–14]. DJ-1 which was recently detected in lung cancer induced MPEs interacts with SOD1 and the Nuclear factor erythroid 2 (NF-E2)-related factor 2/Kelch-like ECH-associated protein 1 (Nrf2/Keap1) pathway in conditions of oxidative stress. Relevant to this is the fact that Sesn2 has additionally been shown to prevent oxidative stress by the activation of Nrf2 and promotion of p62-dependent autophagic degradation of Keap1 [16]. Therefore the potential secretion of Sesn2 in MPEs may be another layer of antioxidant defense in conjunction with counterparts reported in previous studies. Further investigation is required to conclude as to the validity of our hypothesis.

4. Conclusion

To the best of our knowledge, this is the first study to evaluate the levels of Sesn2 in the pleural fluid of patients with PEs and to report its utility as a biomarker for differentiating MPEs from BPEs. Moreover, our study is the first to suggest that Sesn2 is potentially secreted locally in the pleural cavity, a finding that warrants further investigation.

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