High Expression of PEA15 Is Associated With Patient Survival in Malignant Pleural Mesothelioma

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Abstract. *Background/Aim: Malignant pleural mesothelioma (MPM) is a rare but very aggressive tumor that is primarily pleural in origin. The 5-year overall survival rate of patients with MPM has not improved despite therapeutic advances. Therefore, biomarker discovery to identify premalignant or early malignant tumors of the mesothelium are crucial. PEA15 is a cytoplasmic protein that is involved in various human malignancies, including MPM. However, the clinicopathological involvement of PEA15 in MPM has not yet been documented. Materials and Methods: The Oncomine database and GEPIA2 platform were used to analyze PEA15 mRNA expression and patient survival in patients with MPM. Results: PEA15 was found to be significantly up-regulated in MPM, and this up-regulation inversely correlated with prolonged patient survival. Further, PEA15 expression was found to be increased in other cancer tissues without affecting overall survival. Conclusion: PEA15 may represent a new potential prognostic biomarker in MPM patients.*

Malignant mesothelioma is an aggressive tumor arising primarily from pleural or peritoneal cavities. Up to 80% of malignant mesotheliomas are pleural in origin and are defined as malignant pleural mesotheliomas (MPM). Asbestos remains the major cause of MPM and presenting clinically 10-40 years after first exposure. Due to delayed diagnosis, the overall survival in MPM remains dismal, and better prognostic biomarkers are still required (1). Proteomics is a technique for studying cellular

protein expression and has been widely applied to identify biomarkers for various human malignancies. Two-dimensional gel electrophoresis (2-DE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) are major techniques for proteomics. The combinatorial approach of 2-DE and LC-MS/MS with western blotting has high throughput and accuracy, and it is useful for comprehensively analyzing human disease proteomes (2). Previously we performed proteomic differential display analysis by using 2-DE and LC-MS/MS of three human MPM cell lines compared to a normal human pleural mesothelial cell line (3). Our proteomic analysis showed that only one protein spot level was different between the MPM cell lines and the normal pleural mesothelial cell line, and this protein spot was identified as phosphoprotein enriched in astrocytes 15 (PEA15). We then validated the expression of PEA15 by western blot analysis. We confirmed that MPM cell lines have significantly higher levels of PEA15 compared to a normal pleural mesothelial cell line and normal pleura tissues from patients. These findings suggested that the expression of PEA15 could be useful clinically to identify asymptomatic premalignant or early malignant tumors of mesothelium and may provide potential therapeutic targets for it. However, the clinicopathological relevance of PEA15 expression in MPM has not been yet documented. This study aimed to clarify the clinical significance of PEA15 expression in mesothelioma patients by using several bioinformatics platforms. In addition, PEA15 mRNA expression and its clinical significance in other cancers were assessed using the bioinformatics tool (4). Finally, a literature review was conducted to investigate the involvement of PEA15 and the underlying mechanisms in various malignancies using effective search engines.

Materials and Methods

mRNA expression and survival analysis of PEA15 in malignant pleural mesothelioma. To explore PEA15 mRNA expression level in MPM, the Oncomine database was used (https://www.oncomine.org/resource/login.html; last access: June 1, 2021). In the Oncomine database, the gene name "PEA15" was entered. The differential gene analysis module (cancer vs. normal analysis) was selected to retrieve
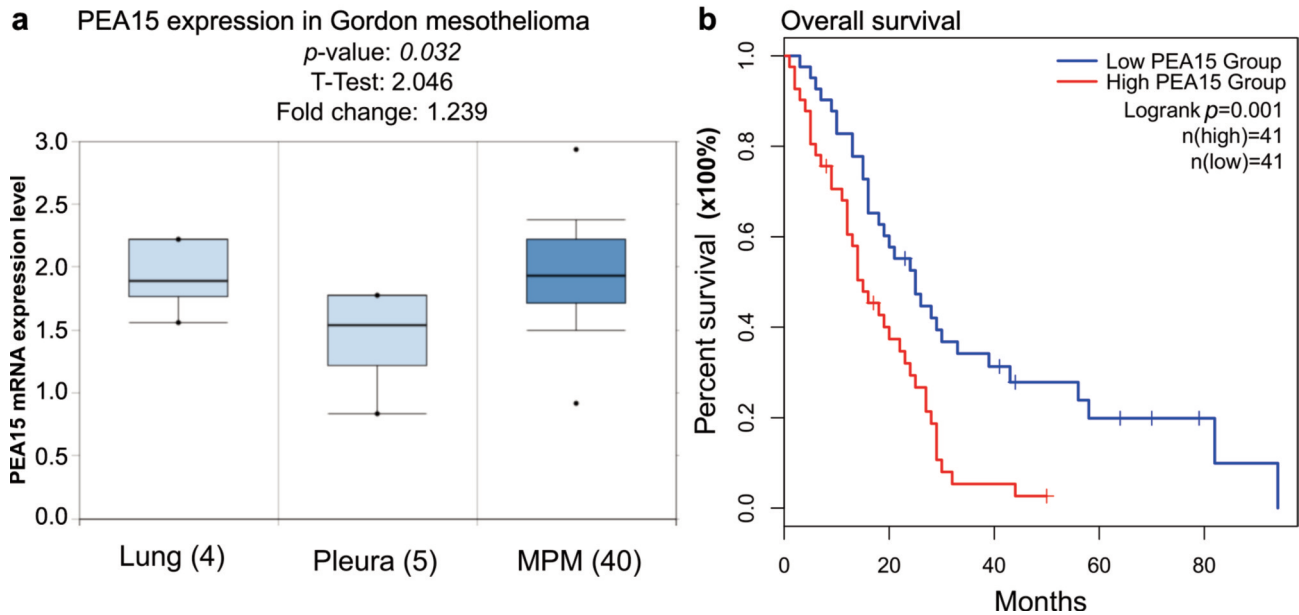
Figure 1. *mRNA expression and Kapan-Meier survival analysis of PEA15 in mesothelioma patients. (a) The mRNA expression level analysis of PEA15 in MPM tissues. The boxplots were downloaded from the Oncomine database. The light blue box represents normal lung (n=4) and pleural tissues (n=5), wherein the dark blue box represents MPM tissues (n=40). p=0.05 was regarded as statistically significant. (b) The overall survival analysis of PEA15 was performed by using the GEPIA2 platform. The overall survival curve of mesothelioma patients compared between a high PEA15 expression group (red line) and a low PEA15 expression group (blue line). p=0.01 was regarded as statistically significant.*

the results. This analysis presented a series of mesothelioma studies and related PEA15 expression in cancer and normal tissues. The filters were set as follows: i) Gene: PEA15. ii) Analysis type: cancer vs. normal analysis. iii) Cancer type: MPM. iv) Sample type: clinical specimen. v) Data type: mRNA. vi) Threshold settings: $p=0.05$. The survival analysis was performed to investigates the PEA15 gene in mesothelioma by using the Gene Expression Profiling Interactive Analysis (GEPIA2) platform from The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) databases. The median was selected for group cut-off criteria. $p=0.01$ was considered to indicate a statistically significant difference.

Evaluation of PEA15 expression in different cancerous tissues. PEA15 mRNA expression levels in other cancer tissues were explored from the TCGA and GTEx databases, using the GEPIA2 platform. The mRNA expression cut-off criteria were selected as follows: LogFC cut-off=1.5; p-value cut-off=0.05; datasets=mesothelioma; and matched normal data=match TCGA normal and GTEx. The survival analysis of PEA15 in different cancerous tissues was performed using the GEPIA2 platform. $p=0.01$ was considered to indicate a statistically significant difference.

Involvement of PEA15 in cancers: a literature review. Two databases, namely PubMed and Scopus, were screened for relevant articles and were limited to articles published in English. Data were extracted from the databases on June 1, 2021. The formulated search strategy was used in the databases: (PEA15 [MeSH Terms]) OR (PEA-15 [MeSH Terms]) AND (cancer [MeSH Terms]). Peer-reviewed studies were considered for the last 5-years, and after a comprehensive analysis, 11 studies were selected (5-15).

Results

High levels of PEA15 expression are inversely correlated with prolonged patient survival in MPM. To explore the mRNA expression levels of PEA15 in MPM tissues, the Oncomine database was used. Boxplots of the PEA15 associated with MPM were downloaded from the Oncomine platform. The results demonstrated that PEA15 was significantly up-regulated in MPM tissues in comparison to normal pleural and lung tissues ($p=0.05$) (Figure 1a). The Kaplan–Meier survival plot was generated using the GEPIA2 platform, and the overall survival status was analyzed. Elevated expression levels of PEA15 were found to be inversely correlated with prolonged patient survival ($p=0.01$) (Figure 1b).

Increased PEA15 expression observed in different cancer without affecting patient survival. To identify PEA15 mRNA expression and survival status in other cancerous tissues, TCGA datasets were analyzed by using the GEPIA2 platform. Boxplots of the PEA15 in different cancer tissues were downloaded from the GEPIA2. The results demonstrated that PEA15 mRNA levels were up-regulated in cholangiocarcinoma, glioblastoma multiforme, pancreatic ductal adenocarcinoma, cutaneous melanoma, and thymoma in comparison to normal tissues ($p=0.05$) (Figure 2). We then analyzed whether the increased PEA15 expression levels affected the patient survival on those cancers. The
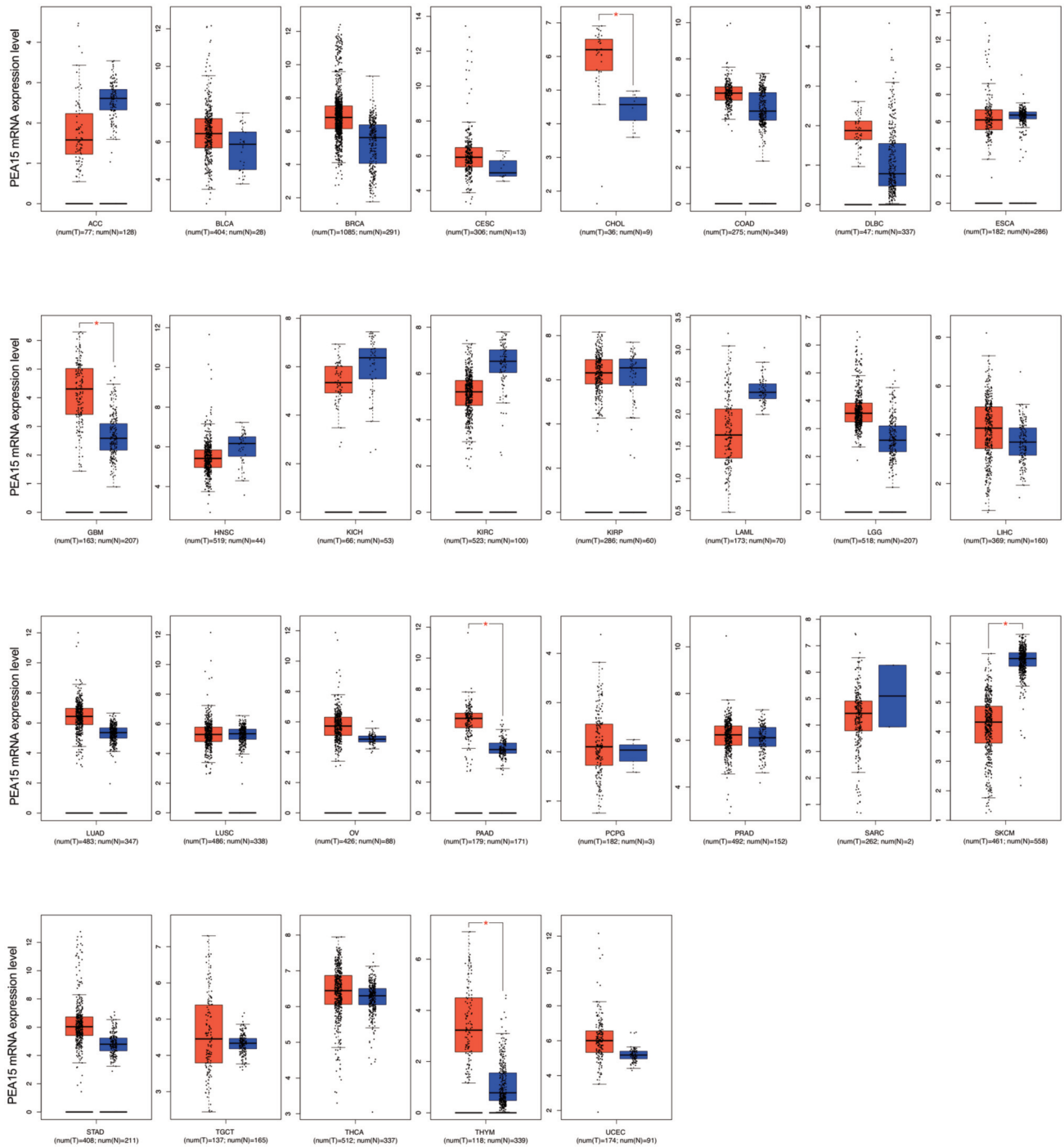
Figure 2. *The mRNA expression level analysis of PEA15 in different cancer tissues. The boxplots were downloaded from the GEPIA2. The red boxes represent the expression levels in cancerous tissues. In contrast, the blue boxes represent the expression levels in normal tissues. p=0.05 was regarded as statistically significant. ACC: Adrenocortical carcinoma; BLCA: bladder urothelial carcinoma; BRCA: breast invasive carcinoma; CESC: cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL: cholangiocarcinoma; COAD: colon adenocarcinoma; DLBC: lymphoid neoplasm diffuse large B-cell lymphoma; ESCA: esophageal carcinoma; GBM: glioblastoma multiforme; HNSC: head and neck squamous cell carcinoma; KICH: kidney chromophobe; KIRC: kidney renal clear cell carcinoma; KIRP: kidney renal papillary cell carcinoma; LAML: acute myeloid leukemia; LGG: brain lower grade glioma; LIHC: liver hepatocellular carcinoma; LUAD: lung adenocarcinoma; LUSC: lung squamous cell carcinoma; OV: ovarian serous cystadenocarcinoma; PAAD: pancreatic ductal adenocarcinoma; PCPG: pheochromocytoma and paraganglioma; PRAD: prostate adenocarcinoma; READ: rectum adenocarcinoma; SARC: sarcoma; SKCM: skin cutaneous melanoma; STAD: stomach adenocarcinoma; TGCT: testicular germ cell tumors; THCA: thyroid carcinoma; THYM: thymoma; UCEC: uterine corpus endometrial carcinoma; UCS: uterine carcinosarcoma; UVM: uveal melanoma.*
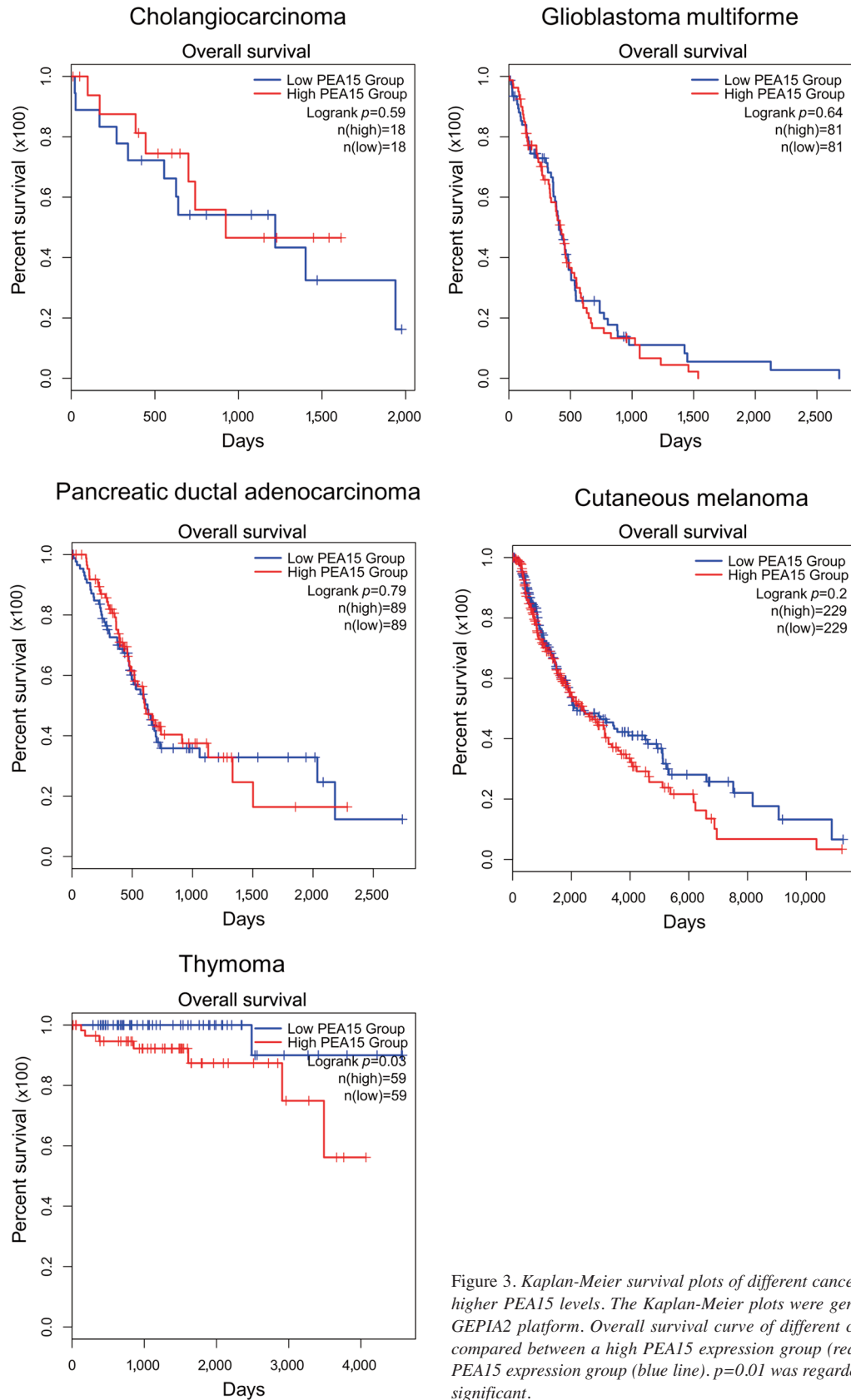
Figure 3. *Kaplan-Meier survival plots of different cancerous tissues with higher PEA15 levels. The Kaplan-Meier plots were generated using the GEPIA2 platform. Overall survival curve of different cancerous tissues compared between a high PEA15 expression group (red line) and a low PEA15 expression group (blue line). p=0.01 was regarded as statistically significant.*

Table I. *Involvement of PEA15 in different cancers.*

A, Tumor suppressor

Author, year	Effects	Cancer types
Mohammad *et al*., 2016	Promotes apoptosis and inhibits cell growth through interacting with protein phosphatase 4	Breast cancer

B, Tumor promoter

Author, year	Effects	Cancer types
Geng *et al*., 2016	Promotes cell proliferation, migration, and inhibition of apoptosis; inhibits tumor suppressor miRNA-132 expression	Glioma
Kawakami *et al*., 2017	Promotes cell growth and metastatic invasion	Lung cancer
Quintavalle *et al*., 2017	Promotes expression of genes associated with metastasis and invasion; high expression attenuates the antitumoral effect of sorafenib by inhibiting caspase-3/7 activity	HCC
Tang *et al*., 2019	Promotes cell proliferation, invasion, and metastasis through the pathway associated with ERK/MAPK; promotes EMT; high expression is inversely related with favorable clinical feature	Colorectal cancer
Jiang *et al*., 2019	Promotes cell growth by increasing cyclin D1 expression through the ERK pathway; inhibits apoptosis, and promotes cisplatin resistance	Gastric cancer
Dilruba *et al*., 2020	Contributes to promoting cisplatin resistance through interacts with ERK pathway	Ovarian cancer
Wang *et al*., 2020	Promotes the proliferation of cancer cells through interacts with Zinc finger protein 70	Ovarian cancer
Luo *et al*., 2020	Promotes cell proliferation and inhibits apoptosis by decreases Bcl2 and Bax and cleaved caspase-3; negatively regulates the expression of tumor suppressor miRNA-212	Ovarian cancer
Wang *et al*., 2021	Regulates mesenchymal modalities of cancer cells	Colorectal cancer
Wu *et al*., 2021	High expression level associated with the tumor staging and grading, vascular invasion, and tumor multiplicity	HCC

ERK: Extracellular-regulated kinase; MAPK: mitogen-activated protein kinase; EMT: epithelial-mesenchymal transition; Bcl2: B-cell lymphoma; Bax: Bcl-2 associated X-protein; HCC: hepatocellular carcinoma.

Kaplan-Meier survival plots showed no significant correlation with high PEA15 expression on those cancers (p=0.01) (Figure 3).

PEA15 functions on different cancer depended on its phosphorylation status. A literature review was performed to investigate the involvement of PEA15 and the underlying mechanisms in various malignancies using effective search engines. The search strategy resulted in 45 potentially eligible studies, with 16 in PubMed and 29 in Scopus. After removing the duplicates, the first screening resulted in 31 studies singled out for evaluation. After a comprehensive analysis, 11 studies were chosen based on the criteria which were set. The effects of PEA15 in different cancers were explored, as shown in Table I. In brief, PEA15 was found to play both tumor-suppressive and tumor-promoting functions. The dual functions of PEA15 in various cancers are dependent on its phosphorylation status. Only unphosphorylated PEA15 was found to act as a tumor suppressor. In contrast, phosphorylated PEA15 promotes cancer progression through various pathways, including extracellular-receptor kinase (ERK), AMP-activated protein kinase (AMPK), and mitogen-activated protein kinase (MAPK). Aberrant activation of these pathways by PEA15 has been associated with cisplatin resistance in various cancers.

Discussion

In the present study, PEA15 mRNA expression and Kaplan-Meier survival were analyzed in MPM patients using bioinformatics platforms. The mRNA expression level of PEA15 was significantly up-regulated in MPM, and this up-regulated PEA15 expression was inversely correlated with prolonged patient survival. In addition, PEA15 mRNA expression and Kaplan-Meier survival graphs in various cancers were generated by using the GEPIA2 platform. We found high PEA15 expression levels in patients with cholangiocarcinoma, glioblastoma multiforme, pancreatic ductal adenocarcinoma, cutaneous melanoma, and thymoma. However, high PEA15 expression levels in these cancers do not significantly impact overall patient survival. Therefore, the data from our present analysis suggest that PEA15 may be a promising potential biomarker for MPM.

Although the biological involvement of PEA15 in MPM pathogenesis is not yet fully understood, PEA15 is a

cytoplasmic protein and has the potential to direct cell phenotypic modulation. PEA15 has at least two distinct functions within the cell, controlling the localization of ERK1/2 and regulating apoptosis. The level of PEA15 expression thus influences cell survival and proliferation, two key determinants of carcinogenesis. Intriguingly, in tumor biology, PEA15 seems to play contradictory roles as a tumor suppressor or oncogene. The exact mechanisms by which PEA15 drives the outcome one way or other remain unclear. It has been suggested that the phosphorylation status of PEA15 determines its biological functions in the process of tumor development (16). Only unphosphorylated PEA15 has been associated with tumor-suppressor functions. In that scenario, PEA15 interacts with ERK to prevent ERK nuclear translocation and activation of nuclear targets. In contrast, phosphorylation blocks ERK binding to PEA15 and prevents apoptosis. These effects lead to increased cellular transcription, proliferation, invasion, and metastasis (16). This could lead to speculation that the tumorigenic role of PEA15 observed in MPM may be due to its phosphorylation. However, the other possible mechanisms cannot be ruled out. Meanwhile, recent investigations have shown that protein expression profiles are different between MPM and other cancer cell lines. Cytohistological differences of MPM cells compared to other cancers may explain this phenomenon (17). The alteration of PEA15 levels may, therefore, be dependent on cell types. Further studies are needed to clarify the exact mechanisms by which PEA15 promotes the progression of MPM.

Conflicts of Interest

The Authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' Contributions

All Authors contributed to the study's conception and design. Data collection and analysis were performed by Shajedul Islam, Takao Kitagawa, and Yasuhiro Kuramitsu. Shajedul Islam wrote the first draft of the manuscript, Takao Kitagawa and Yasuhiro Kuramitsu commented on previous versions of the manuscript. All Authors read and approved the final manuscript.

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