ASF1B May Regulate the Tumor Microenvironment and Epithelial-mesenchymal Transition in Malignant Mesothelioma to Induce the Differentiation of Sarcomatoid Phenotype as a Prognosis Target

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Abstract

Introduction

Malignant mesothelioma (MM) is a rare malignant tumor with a poor survival. However, few markers are verified that related to sarcomatoid differentiation or further stratification of prognosis.

Methods

We analysis the significance of ASF1B expression in MM by analyzing the public dataset from TCGA, GEO and Oncomine database. Furthermore, we investigated the biological function of ASF1B in immune microenvironment and the effect of ASF1B in survival, gene ontology enrichment, GSEA enrichment and related microRNA.

Results

ASF1B expression was significantly higher in MM by GEO data compared to normal lung tissue ($p < 0.0001$). This study provides evidence that the increased expression of ASF1B is significantly associated with poor prognosis and inhibitory immune cell infiltration in patients with MM and highlight that ASF1B could be used as a novel predictive biomarker for the prognosis of MM which is related to the differentiation of sarcomatoid phenotype.

Conclusion

ASF1B May Regulate the Tumor Microenvironment and Epithelial-mesenchymal Transition in Malignant Mesothelioma to Induce the Differentiation of Sarcomatoid Phenotype as a Prognosis Target. Further researches need to be conducted to figure out how exactly the ASF1B affect the microenvironment.

1. Introduction

The mesothelium is a cell monolayer that is spread over the surface of serosal organs and cavities. The primary function of mesothelium is to protect tissues and organs from damage or infection. The tumor of the mesothelium is called a malignant mesothelioma (MM). It is a rare malignant tumor with a median survival of 6 to 12 months.¹ The incidence of MM has increased constantly and is estimated to increase continuously around the world. The exposure to asbestos is the main cause of this devastating disease. With the recent developmental works such as reconstruction and restoration, exposure to asbestos is evitable in some countries where mesothelioma will remain a health burden until 2030.²

According to the present clinical and laboratory studies, Mesothelioma is an aggressive cancer that occurs in the mesothelium and is strongly associated with asbestos exposure. Fibrous mineral erionite, carbon nanotubes and gene mutations, radiation and Simian Virus 40 are considered to be the most hazardous factors that contribute to the development of mesothelioma.³

Histologically, mesothelioma can be classified into three subgroups which include epithelioid, sarcomatoid and biphasic types. The type of epithelioid or sarcomatoid is morphologically characterized by the presence of polygonal or spindle-like cells. The underlying mechanism for the development of histologically different mesothelioma types is yet to be elucidated. The current MM diagnosis includes two positive and two negative histochemical markers. However, there is little data available on markers that could help to distinguish sarcomatoid types or further stratify prognoses amongst histologic variants which makes diagnosis even more difficult. In addition to the definition of multiple morphological patterns of these subtypes, the primary lung cancer and pleural metastases are similar in clinic presentation and histological appearance which make MM diagnosis even more challenging.⁴ Epithelial-mesenchymal transition (EMT), which is related to tumor proliferation and disease progression, represents a reversion in embryological development.⁵ Several studies have proved that EMT plays a significant role in the morphological characteristics of malignant mesothelioma and eventually lead to the development of sarcomatoid patterns.⁵⁻⁶ In order to enhance the diagnostic accuracy, it is important to elaborate the function of oncogenic genomic changes in MM as predictive biomarkers and future therapeutic targets.

Aberrant expression of genes, including downregulation or upregulation, is correlated to the changes in DNA methylation and abnormal histone modification. The deregulation of chromatin regulators, including histone variant proteins, histone chaperone proteins, histone-modifying enzymes, effector proteins, and chromatin-remodeling proteins have been reported to be involved in the development, progression and metastasis of cancer.⁷

Histone H3–H4 chaperone anti-silencing function 1 (ASF1) ASF1 is a major histone chaperone protein which plays a role in the progression of chromatin-based cellular DNA replication, repair of DNA damage, and regulation of transcription. Two paralogs of ASF1 exist which include ASF1A and ASF1B. ASF1A specifically contributes to cell senescence and DNA repair, while as ASF1B predominantly participates in cell proliferation. Till date there is not any study which reports the correlation between the expression of ASF1B and mesothelioma.⁸

Consistently, this study was designed to evaluate the effect of ASF1B gene expression on the survival prognosis of MM patients. Herein, bioinformatics analysis was performed based on high-throughput sequencing data from TCGA and the results were verified in GEO and Oncomine to identify how ASF1B gene expression impacts on the survival prognosis of MM patients.

2. Materials And Methods

2.1. Raw data download and preprocessing

The gene expression raw data and clinical data of 84 MM patients were downloaded from the Cancer Genome Atlas (TCGA) data portal(<https://portal.gdc.cancer.gov/>).⁹ Additionally, 45 tumors and 3 normal tissues, 55 tumors along with paired normal tissues, 38 tumors and 9 normal tissues were respectively

download from Gene Expression Omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo/>) (GSE112154 in Illumina HumanHT-12 V4.0 expression beadchip¹⁰, GSE51024 in Affymetrix Human Genome U133 Plus 2.0 Array¹¹, GSE42977 in Illumina HumanRef-6 v2.0 expression beadchip¹²), famous for the high-throughput microarray and next-generation sequence functional genomic data sets. The detail expression data can be obtained in the supplement table 1¹³. Total of 99 samples of mesothelioma were downloaded from Lopez-Rios' s studies in ONCOMINE, which known as a cancer microarray database and web-based data-mining platform A total of 99 samples of mesothelioma were downloaded from Lopez-Rios' s studies in ONCOMINE, which is known as cancer microarray database and web-based data-mining platform.¹⁴.

Most of those data were reserved in the form of expression profiling by array. Most of these data were reserved in the form of expression profiling by array. The R was used for data downloading and selection. (<https://cran.r-project.org/>) (R software, version 3.6.2). Subsequently, we made the table of clinical data of study groups and COX regression with the R software to show our findings. Receiver operating characteristic curve (ROC curve) was plotted by Graphpad prism V7(<https://www.graphpad.com/scientific-software/prism/>). The code can be obtained in the supplement files about R.

2.2. ASF1B expression, methylation and clinicopathological features of MM by GEPIA, UALCAN and cbiportal

The clinical data of 84 patients were used for Cox regression analysis. The survival analysis of ASF1B expression from TCGA database was performed with the GEPIA 2 website. (<http://gepia2.cancer-pku.cn/#index>)¹⁵. In TCGA, there were no paired normal tissues of MM and as such the differentially expressed genes could not be determined.

UALCAN is an interactive web-portal to perform the in-depth analyses of TCGA gene expression data (<http://ualcan.path.uab.edu/analysis.html>)¹⁶. The boxplots were drawn by UALCAN with the correlation between the expression or methylation of ASF1B and different ages of the patients, nodal metastasis status and tumor histology. The cBio Cancer Genomics Portal (cbiportal, <http://www.cbiportal.org/>)¹⁷ was also used to investigate the relationship of mRNA expression with the level of ASF1B methylation.

2.3 Gene ontology enrichment and similar gene mutation

The UniProt-GOA database (<http://www.ebi.ac.uk/GOA/>)¹⁸ was used to perform the GO analysis on ASF1B. Subsequently, the LinkedOmics (<http://www.linkedomics.org/login.php>) as used to find the correlated genes and to identify the downregulated and upregulated genes and present them in the form of a heat map.¹⁹ Furthermore, PPI network was constructed to find the interaction of those hub genes in STRING (<https://string-db.org/>).

Moreover, the related microRNAs have also been detected in LinkedOmics and the survival of MM patients affected by those miRNAs were investigated by the OncomiR (http://www.oncomir.org/oncomir/survival_custom.html)²⁰.

2.4 Analysis of the relative abundance of tumor infiltrating immune cells (TIICs)

The Cibersortx (<https://cibersortx.stanford.edu/index.php>) was used to identify the different immune cells in the tumor microenvironment by contrasting the gene expression in tumor with immune cells in the high and low ASF1B expression groups in 84 TCGA-MM patients and 41 GEO mesothelioma patients according to the deconvolution algorithm.²¹

TIMER (<https://cistrome.shinyapps.io/timer/>)²² is a platform for systematical analysis of immune infiltrates across diverse cancer types. The compose of immune cells in tumors like B cells, CD8+ T cells, CD4+ T cells, Macrophages were evaluated and the correlation of ASF1B expression with immune infiltrates in MM was visualized in TIMER.

2.5 GSEA pathway analysis

Gene set enrichment analysis (GSEA) is commonly used to reveal biological pathways by interpreting gene expression data. In this study, GSEA was used to analyze the differential signaling pathways of the activation of ASF1B low and high expression groups in MM patients. LinkedOmics (<http://www.linkedomics.org/>) is a comprehensive multiomics analysis platform for the TCGA database. The LinkedOmics was used to find the ASF1B correlated genes, including downregulated and upregulated genes, and to verify the pathway enrichment of GSEA. The minimum number of genes (size) was set to 3 and simulation to 500, and $p < 0.05$ is taken as a measure of significant enrichment.

2.6 Statistical analysis

The correlation between ASF1B expression and the clinicopathological parameters was assessed by Pearson’s Chi-square test. The independent indicators related to OS were identified using Cox proportional hazards model, and the hazard ratios (HR) with 95% confidence intervals (CI) were also calculated. The correlation between the ASF1B mRNA expression and the ASF1B DNA methylation level (or the miRNAs expression) was evaluated by linear regression analysis. SPSS 22.0 and R version 3.6.2 were used for statistical analyses. A two-tailed p value less than 0.05 was considered to be statistically significant.

3. Results

Table 1. The relationship between ASF1B expression and some clinicopathological features

ASF1B Expression				
	Total(N=84)	High(N=45)	Low(N=39)	p
Age (year)				
≤65	44(52.4%)	22(48.9%)	22 (56.4%)	0.64
≥65	40(47.6%)	23(51.1%)	17(43.6%)	
Gender				
female	15(17.9%)	8(17.8%)	7(17.9%)	0.79
male	69(82.1%)	37(82.8%)	32(82.1%)	
Stage				
i	10(11.9%)	7(15.6%)	3(7.7%)	0.50
ii	15(17.9%)	6 (13.3%)	9 (23.1%)	
iii	43(51.2%)	24(53.3%)	19(48.7%)	
iv	16(19.0%)	8(17.8%)	8 (20.5%)	
OS				
Dead	72(85.7%)	41(91.1%)	31(79.5%)	0.13
Alive	12(14.3%)	4(8.9%)	8(20.5%)	
Asbestos Exposure				
No	14(16.7%)	7(15.6%)	7(17.9%)	0.77
Yes	70(83.3%)	38(84.4%)	32(82.1%)	
Laterality				
Bilateral	3 (3.6%)	2(4.5%)	1 (2.6%)	0.72
Left	31 (36.9%)	15(33.3%)	16(41.0%)	
Right	50 (59.5%)	28(62.2%)	22 (56.4%)	

3.1 ASF1B expression in normal and tumor tissue

The clinical characteristics, and the Chi-square test results for the comparison of MM with different ASF1B expression is presented in Table 1. The Chi-square test revealed that the malignant mesothelioma was more prevalent in male (82.1%), mainly attacking on the right side (59.5%) and people with asbestos exposure history (83.3%), The incidence ratio of male to female was found to be 4.6:1. Most of patients

were in stage iii (51.2%), however, significant difference was observed in none of those subgroups. ($p>0.05$).

In three GEO databases (GSE112154, GSE51024, GSE42977), ASF1B expression was found to be significantly higher in the MM patients compared to the paired normal lung tissues, normal pleura or peritoneum samples. ($p<0.05$) (Yingjie Jia Figure1A, 1C, 1D).

The receiver operating characteristic (ROC) curve of the test cohort is presented in Yingjie Jia Figure1B. Area under the curve (AUC) was 0.9326 ($p<0.0001$) and the cut-off value was 7.635 (Specificity:87.27%, Sensitivity:95.12%). The specificity and AUC were calculated for the discrimination of mesothelioma from the paired normal controls in GSE51024.

3.2 The relationship between ASF1B expression and some clinicopathological features

In TCGA-MM, there were no compared tissue of mesothelioma so we could not determine the differential expression of ASF1B between normal and tumor tissues. However, the survival analysis was performed, and it was found that in low-expression group, patients tend to have longer OS than high-expression group. ($p<0.05$, Fig 2).

Through COX univariate analysis, it was found that the reduced expression of ASF1B was significantly related to longer overall survival. ($P<0.001$) This means that the low ASF1B expression level is a significant protective factor for the survival. (Table 2). Other clinical information like stage, gender, laterality and histology showed no significant difference.

Previous studies have shown that pathological type is an independent predictor of survival of malignant mesothelioma. The degree of differentiation was also an independent predictor of survival of malignant mesothelioma.²³ Although there is no significant difference ($p>0.05$), our univariate Cox regression analysis indicated that sarcomatoid mesothelioma might be a hazard factor as compared to the epithelioid type ($HR=2.00$, $95\%CI=[0.79, 5.11]$).

A correlation analysis was performed on 84 patients with clinical case characteristics (age of the parents, tumor histology, status of nodal metastasis, clinical stage) using the UALCAN (Fig. 3).

The results showed that the increase in ASF1B expression was significantly correlated with histology (Yingjie Jia Figure3D, 3E, 3F). The results were verified by the data in GEO (Yingjie Jia Figure3E)($p<0.05$), Oncomine (Yingjie Jia Figure3F)($p<0.05$). The results showed that significant upregulation of ASF1B in biphasic mesothelioma and sarcomatoid mesothelioma, which are considered to be more malignant. And this could be one explanation of how ASF1B induces the progression of mesothelioma.

Table 2. COX univariate analyses of clinical characteristics and survival.

Clinicopathologic variable	Univariate analysis	
	HR95%CI	p value
Age ≥ 65 vs. <65	1.25[0.77, 2.05]	0.37
Gender (male vs. female)	1.20[0.64, 2.26]	0.58
TNM stage (II vs. I)	0.55[0.23, 1.31]	0.18
TNM stage (III vs. I)	0.57[0.27, 1.19]	0.13
TNM stage (IV vs. I)	0.54[0.23, 1.26]	0.15
Asbestos exposure (yes vs. no)	1.40[0.74, 2.65]	0.30
Laterality (left vs. right)	0.65[0.39, 1.07]	0.09
Laterality (bilateral vs. right)	1.15[0.35, 3.74]	0.82
Histology (sarcomatoid vs. epithelioid)	2.00[0.79, 5.11]	0.15
Histology (biphase vs. epithelioid)	1.68[0.97, 2.94]	0.07
ASF1B	1.65[1.29, 2.11]	<0.001

H3K9me1 is the most prevalent methylation mark with ASF1B. The correlation between ASF1B methylation level and the clinicopathological features, was investigated and it was found that the degree of ASF1B methylation was lower in 81-100 years old patients (Yingjie Jia Figure4A, 4B)($p < 0.05$). With the cbiportal, it was found that the ASF1B methylation level is negatively related to the ASF1B mRNA ($R = -0.27$, $p = 0.01$), and this result is presented in Yingjie Jia Figure4C.

3.3. GO enrichment, KEGG pathways and comparable mutant genes

The GO enrichment includes three parts-molecular function, biological process, cellular component, and the ASF1B mainly function in histone binding in nucleus and chromatin (Table 3).

MSigDB enrichment analysis was performed for this study, to screen for signaling pathways with major variations caused by the expression of ASF1B ($p < 0.05$). Subsequently, the KEGG pathway analysis by the LinkedOmics platform (Yingjie Jia Figure. 5) was compared with the GSEA(Yingjie Jia Figure 6), and those pathways mainly functioned in the proliferation of cells.

Table 3. GO enrichment on ASF1B

Category	Accession	Term
Cellular Component	GO:0005634	nucleus
	GO:0000785	chromatin
	GO:0005654	nucleoplasm
	GO:0032991	protein-containing complex
Biological Process	GO:0006335	DNA replication-dependent nucleosome assembly
	GO:0006333	chromatin assembly or disassembly
	GO:0030154	cell differentiation
	GO:0006325	chromatin organization
	GO:0001835	blastocyst hatching
	GO:0007275	multicellular organism development
	GO:0007283	spermatogenesis
	GO:0006334	nucleosome assembly
	GO:0042393	histone binding
Molecular Function	GO:0005515	protein binding

The main pathways modulated by ASF1B were mainly related to cell cycle and include chromosome segregation, organelle fission, spindle organization, DNA replication and mitotic cell cycle phase transition. At the same time, ASF1B also could downregulate the process of cargo loading into vesicle, protein activation cascade, response to interleukin-6 and platelet-derived growth factor receptor signaling pathway.

In GSEA between the high and low AFS1B expression datasets to screen out the differentially activated signaling pathways, ASF1B modulated the gene replication, nucleotide excision repair, and base excision repair to name a few.

As revealed by the previous studies, ASF1B in proliferating cells handles the pool of replicative histone H3.1 thereby acting as the prominent histone acceptor/donor during DNA replication²⁴.

3.4 Related genes and their function by STRING

In our analysis, BDH2, KLHL9, C13orf33, CMAH, TM4SF1, TUSC1, BBS12, ANXA8, SLC4A4, SERPING1, LMBRD1, IFITM2, YPEL3, SYNE1, DYNC2LI1, RICH2, CALCOCO1, PIP5K1B, ITLN1, KBTBD3, NPR1 were negatively related to the expression of ASF1B in MM patients,

Meanwhile, CDCA5, RAD54L, STMN1, BIRC5, TROAP, CCNB2, CDCA3, FOXM1, KIFC1, UBE2C, SPC25, PKMYT1, MCM7, KIF18B, ORC1L, SPAG5, MCM2, KIF2C, GINS4, POLD1, AURKB, RAD51 were positively related to ASF1B in MM patients. (Yingjie Jia Figure 7A-7B)

STRING shows that these closely related genes were all function in the process of TATA box binding protein associated factor (TAF), Centromere kinetochore component CENP-T histone fold and core histone H2A/H2B/H3/H4(Yingjie Jia Figure 7C).

We investigated the relative expression of miRNAs and found that hsa-miR-503, hsa-miR-130b, hsa-miR-301b, hsa-miR-196b were positively correlated to the expression of ASF1B and they all could significantly shorten the survival of MM patients. In contrary, hsa-miR-29c, hsa-miR-195, hsa-miR-100, hsa-miR-30d were negatively correlated to the expression of ASF1B and could prolong the OS time. (Yingjie Jia Figure 8)

3.5 Association between ASF1B expression and composition of TILCs

In the database pf TCGA, macrophage M1, macrophage M2, CD8 T cell, T cell follicular helper and regulatory T cell showed a significant increase in ASF1B high-expression group. In contrary, the NK cells resting, monocytes, eosinophils, neutrophils, dendritic cells activated, CD4 T memory resting showed significant decrease under high expression of ASF1B.

In the database of GEO, the regulatory T cells (Tregs), macrophage M1, macrophage M0, dendritic cells resting showed a significant increase in ASF1B high-expression group. In contrary, the B cells naïve, plasma cells, monocytes, dendritic cells activated showed a decrease under the effect of high ASF1B expression (Yingjie Jia Figure 9 and Yingjie Jia Figure 10). The detail proportion of immune cells data can be obtained in the supplement table 2(GEO) and supplement table 3(TCGA).

The results of the present study revealed that under the regulatory influence of ASF1B, the microenvironment changed a lot. These results were verified by GEO and TCGA. Two databases showed that under the effect of ASF1B, the changes of DC cells, Tregs and the subgroup of macrophages indicated the immune suppression in the tumour microenvironment. More than that, many other immune cells need further investigated.

4. Discussion

The present study revealed that the incidence of malignant pleural mesothelioma is comparatively higher in male (82.10%), generally present on the right side (59.5%) and in people with asbestos exposure history (83.3%). The prevalence of MM has sharply increased over the last 50 years, with a ratio of 4.6:1 from male to female. These findings in agreement with the results of previous studies.

To find out the subtypes of mesothelial, epithelial, or sarcomatous distinction in malignant cells, pathological diagnosis was done with the help of immunohistochemical analysis [37]. The reported

diagnostic yield from CT guided biopsy ranged from 60% to 85% with multiple attempts and the highest yields were acquired by open or thoracoscopic pleural biopsy. [38]

Since MM has a partial fibroblastic phenotype within EMT, this can partly explain why it is a highly invasive and chemoresistant cancer, and can be utilized to distinguish epithelioid from sarcomatoid MM²⁵. In this regard, it is possible to consider the epithelioid and sarcomatoid histological forms of MPM as E- and M-parts of the EMT axis, considering the biphasic histotype as an intermediate. Like cells undergoing EMT, sarcomatoid type have been recognized to have a number of mesenchymal-like features such as enhanced ability to migrate, high invasiveness, increased resistance to apoptosis, and substantially increased extracellular matrix components.²⁶

In tumors, a significant proportion of immune cells secreting different cytokines and chemokines to perform paracrine on nearby carcinoma cells is measurable in the tumor microenvironment to induce inflammation. Inflammation in tumors is a powerful inducer of EMT. A combination of these paracrine signals contributes to the EMT of carcinoma cells leading to the promotion of the tumor metastasis.

Tumor associated macrophages (TAMs) are probably the most abundant cell type within immune microenvironment of solid tumors, accounting for up to 50% of tumor mass²⁷. Several studies have revealed macrophages to induce EMT via secretion of a unique cluster of cytokines and chemokines such as TGF β , TNF, CSF, CSF1, GM-CSF, CCL18. The secretion of these cytokines and chemokines triggers EMT and eventually leading to the promotion metastases. Furthermore, cyclooxygenase 2 (COX2), prostaglandin E2 (PGE2) and β -catenin are activated by IL-6 secreted by TAMs. This in turn triggers the activation of β -catenin signaling pathways, leading to an active EMT.

Additionally, studies have proved that Tregs induce TGF- β -mediated EMT and subsequent metastasis of several types of tumor cells such as melanoma and hepatocellular carcinoma and also accelerates the radiation-induced pulmonary fibrosis.²⁸

Till date there is not a single study that reports the relationship between dendritic cell and EMT in mesothelioma. Nonetheless, it has been proved that dendritic cell take part in the EMT in some specific cancer cells such as breast cancer cells.^{25,29} Furthermore, DC phenotype and its production of anti-cancer cytokine IL-12 could trigger changes in the microenvironment of colon cancer.

ASF1 is the evolutionarily most conserved histone H3/H4 chaperone protein from yeast to humans. It effects the heterochromatin silencing and is involved in multiple functions related to chromatin. Its chaperones contribute to chromatin functions by linking histone H3/H4 to DNA. Additionally, it acts as an essential cofactor in histone acetylation on certain histone H3/H4 residues.³⁰

ASF1B is a member of the H3/H4 family of histone chaperone proteins, and functions similarly to ASF1. Tousled-like kinase family of cell cycle-regulated kinases are its downstream molecules. ASF1B also play a key role in modifying the chromatin nucleosome structure by maintaining continuous supply of the histones at nucleosome assembly sites. In the present study, it was found that ASF1B may change the

tumor microenvironment and changes the percentage of the macrophage and some other immune cells such as dendritic cell, monocyte and T cells regulatory (Tregs).

In a previous study, sarcomatoid MM was more likely found to have an M2-like protumor phenotype than epithelioid MM. Our results indicate that high-ASF1B group with higher inhibitory immune cell infiltration (Tregs and macrophage, etc) may result in the phenotype like biphasic mesothelioma or sarcomatoid mesothelioma with EMT, which is related to poor overall survival outcomes and extent of malignancy.

Moreover, we investigated the relative expression of miRNAs and found that hsa-miR-503, hsa-miR-130b, hsa-miR-301b, hsa-miR-196b were positively correlated to the expression of ASF1B and they all could significantly shorten the survival of MM patients. In contrary, hsa-miR-29c, hsa-miR-195, hsa-miR-100, hsa-miR-30d were negatively correlated to the expression of ASF1B and could prolong the OS time. Previous reports have indicated that miR-503-5p is related to the proliferation and invasion of various cancer cells. It was recently found to regulate EMT and also affected the metastasis and prognosis of hepatocellular carcinoma.³¹

Until now, the relationship between hsa-miR-29c, hsa-miR-30 and malignant mesothelioma has been extensively studied. In epithelial mesothelioma, miR-29c* levels have been documented to be elevated relative to sarcomatoid MM. In addition, the miR-29 family itself has been associated with EMT and the expression of B7-H3 proteins, suppressing mesothelioma immune escape.^{32,33} Besides, hsa-miR-30c has been found to be correlated with survival in sarcomatoid tumors.³⁴ However, other miRNAs like hsa-miR-195, hsa-miR-100 have not been fully investigated in the mesothelioma. In the present study, it was found that those miRNAs which were closely related to the ASF1B could affect the survival of MM patients, which needs to be explored further.

The microRNAs take a fundamental part in the regulation of gene expression. Histone modifications, meanwhile, could also modify microRNAs. While numerous studies have been devoted to exploring the miRNA-regulated EMT and epigenetic regulations, their synchronized effect has not been thoroughly examined. To decide if there is a combinatorial action of ASF1B with histone modification and miRNA regulations to mediate the microenvironment and the EMT process, further research endeavors are urgently required.

Conclusion

Collectively, ASF1B could be a predictive factor for mesothelioma patients with the sensitivity of 95.12% and specificity of 87.27%. The expression level of ASF1B is negatively related to the survival of MM patients, which could be considered as a hazardous factor in MM patients. Moreover, ASF1B could mediate the tumor immune infiltrating microenvironment, which may lead to EMT and eventually causing increase in the incidence of biphasic or sarcomatoid mesothelioma, rather than the epithelioid phenotype.

Declarations

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Conflicts of interest/Competing interests

The authors declare no potential conflict of interest such as employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. None of these authors is a member of the editorial board.

Consent for publication

Not applicable

Ethics approval and consent to participate

Not applicable

Availability of data and material (data transparency)

Supplementary Tables could be obtained in the Supplementary Material for detail information about the ASF1B expression in different database.

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Authors' contributions

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Figures

Figure 1

ASF1B in GSE112154, GSE51024, GSE42977 and ROC curve. * $p < 0.01$, ** $p < 0.001$, *** $p < 0.0001$

Figure 2

The overall survival and disease free survival in TCGA

Figure 3

A. Expression of ASF1B in MM based on individual cancer stages. B. Expression of ASF1B in MM based on nodal metastasis status. C. Expression of ASF1B in MM based on patient's age, D. Expression of ASF1B in MM based on tumor histology, E. Expression of ASF1B in MM with different tumor histology in GSE42977, F. Expression of ASF1B in MM with different tumor histology in Lopez Rios MM statistics.

* $p < 0.01$, ** $p < 0.001$, *** $p < 0.0001$

Figure 4

A. Promoter methylation level of ASF1B in MM based on stage, B. Promoter methylation level of ASF1B in MM based on patient's age, C. The correlation of methylation level of ASF1B and mRNA of ASF1B.

Figure 5

The KEGG analysis of ASF1Bin linkedOmics.

Figure 6

The KEGG analysis of ASF1Bin LinkedOmics

Figure 7

A. ASF1B positively related genes B. ASF1B negatively related genes C. Functions of ASF1B closely related genes in STRING

Figure 8

The overall survival affected by ASF1B related microRNA

Figure 9

The immune cells in tumor microenvironment in high-ASF1B group and low-ASF1B group

Figure 10

The immune cells in tumor microenvironment in high-ASF1B group and low-ASF1B group in principal component analysis(pca)

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