Expression changes in the fibulin family in gastric cancer: A Systematic Review

Running title: Expression changes in the fibulin family in gastric cancer

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Abstract

Background: Gastric cancer (GC) is one of the widespread gastrointestinal tumors in the worldwide, with mortality rates among the highest. In the world, we face more than one million newly diagnosed stomach cancer patients every year. Various factors closely associated with formation of GC. One of these factors is the change in the level of components of the extracellular matrix in the microenvironment of the stomach tumor. Fibulins are one of the secreted glycoproteins of extracellular matrix, which are known as matrix organizers. The purpose of this study is to investigate the changes in fibulins in gastric tumor tissue compared to control samples.
Methods: In this systematic review, a search was conducted on June 21, 2024, in alignment with the guidelines set forth by the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement. Scopus, PubMed, and Web of Science databases were searched for articles that examined FBLN gene family and protein expression in patients with gastric cancer and gastric cancer cell lines.
Results: A total of 853 gastric cancer tumor samples, ranging from 8 to 197 per study, were analyzed across eight studies published between 2008 and 2023, all conducted in China. Several gastric cancer cell lines were also included, such as AGS, Kato III, MKN28, MKN45, SNU1, SNU16, NCI-N87, MGC-803, BGC-823, SGC-7901, and HGC27. Tumor size was reported in 4 studies, while histopathological grade and lymph node metastasis were each evaluated in 4 studies.
Conclusion: The downregulation of Fibulin-1 and Fibulin-2 supports their potential tumor-suppressive roles in gastric cancer, while the upregulation of Fibulin-5 is associated to tumor progression and worse prognosis, making it a candidate biomarker for aggressive disease.

Keywords: Gastric cancer, Fibulin1, Fibulin2, Fibulin5

Introduction

Gastric cancer (GC) represents one of the most prevalent forms of malignancy and poses a considerable public health challenge globally (1, 2). It is the fourth leading cause of cancer-related mortality and ranks fifth in terms of incidence (1, 3). Early-stage GC often lacks prominent clinical symptoms, resulting in a diagnosis that typically occurs at more advanced stages (3). Surgery as a standard treatment for GC has substantial effectiveness in its treatment. However, the recurrence rate in GC patients after surgery is high, and the survival rate of patients has not improved significantly (1, 4). The formation and development of GC is a multi-stage process, and various biological factors are involved in it (5, 6).

The external environment and factors are significantly influencing the growth and development of GC cells (7). The extracellular matrix, which constitutes the non-cellular component of all tissues, undergoes regeneration in the context of gastric cancer, leading to alterations in the levels of its constituent components (8). Among these components, the fibulin family of proteins is notable, typically existing as homodimers. Fibulins are essential for the formation and stabilization of elastic fibers, basement membranes, and connective tissue (7).

The fibulin protein family constitutes a significant element of the extracellular matrix and is typically found as a homodimer. These proteins are essential for the development and maintenance of elastic fibers, basement membranes, and connective tissue. (9, 10).

The fibulin family, which includes fibulin1 through fibulin8, consists of secreted glycoproteins that are key components of the extracellular matrix (ECM). The first member identified, fibulin1 (FBLN1), is encoded by a gene situated on chromosome 22p13 in humans and produces a protein weighing between 90 and 100 kilodaltons.(7, 11, 12). FBLN1 is a versatile protein within the ECM, playing a significant role in the structure and function of the basement membrane (13, 14). It contributes to ECM regeneration in tissues and binds to a variety of ECM constituents, including fibronectin, laminin-1, angiogenin, tropoelastin, versican, fibrinogen, proteoglycans, and nidogen. Furthermore, FBLN1 has been identified in plasma (14-17). Its numerous interactions with ECM elements allow it to regulate growth, adhesion, signaling, proliferation, and motility (8, 15). Notably, variations in FBLN1 levels have been linked to several pathological conditions, including different types of cancer, where it has been proposed to act as both an oncogene and a tumor suppressor (7).

Fibulin2 (FBLN2) is a prominent member of the fibulin family (18). This glycoprotein, which is secreted into the extracellular matrix, is situated between elastin nuclei and microfibrils, contributing to the stabilization of the extracellular matrix (18, 19). Furthermore, FBLN2 plays a crucial role in regulating extracellular-intracellular signaling mediated by the extracellular matrix through integrin interactions (18).

Fibulin5 (FBLN5) is a significant member of the fibulin family, involved in cell adhesion, motility, and the association of cells with the extracellular matrix (ECM) (9). Studies have shown that FBLN5 can exhibit contradictory functions, acting as either an inhibitor or a promoter of tumor cell growth, depending on the tumor type and the specific characteristics of the cancerous tissue (20). Moreover, FBLN5 is essential for the regulation of cell growth, metastasis, and tumorigenesis, and it possesses prognostic potential as a tumor suppressor (9, 21).

Given the complex roles of fibulins in cancer biology, this study aims to elucidate the specific contributions of FBLN1, FBLN2, and FBLN5 in gastric cancer progression. By focusing on these proteins, we hope to uncover new insights into their regulatory mechanisms and assess their potential for prognosis and therapeutic targets. This research not only enhances our understanding

of fibulin's role in GC but also highlights the importance of ECM components in cancer development and treatment strategies.

Methods

Information sources

To ensure the integrity of our systematic review, we implemented a thorough process for eliminating duplicates. Initially, we gathered a total of 43 studies from three databases: PubMed (n = 11), Scopus (n = 11), and Web of Science (n = 21). After compiling the studies, we used EndNote as the reference management software to identify and remove duplicate articles. Each study was then cross-verified manually to ensure that all duplicates were eliminated. Following this process, a total of 28 unique articles remained for further screening (Supplementary table) (Figure 1).

Search strategy

A comprehensive search was conducted on June 21, 2024, in alignment with the guidelines set forth by the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement. The search yielded results based on the following keywords: (stomach cancer (Title/Abstract) OR gastric cancer (Title/Abstract) OR gastric carcinoma (Title/Abstract) OR stomach neoplasms (Title/Abstract) OR gastric neoplasms (Title/Abstract) OR stomach carcinoma (Title/Abstract)) AND (Fibulin (Title/Abstract) OR FBLN (Title/Abstract)).

Selection process

The initial assessment was carried out by two reviewers, Jafari and Mahdizadeh, who independently evaluated the titles and abstracts of the articles until February 2024. Following this, the full texts were reviewed according to the specified inclusion and exclusion criteria. Each reviewer then examined all articles separately and later exchanged their selected studies. In situations where differing opinions emerged, particularly with articles featuring microarray analysis, the reviewers would collaboratively reassess the articles to reach an agreement.

Eligibility criteria

Studies must investigate the effects of gene or protein expression of the fibulin family in GC cell lines.

Studies must include human and mouse cancer samples related to GC.

Articles must be published in peer-reviewed journals.

Studies should report original research data, including experimental findings, rather than relying solely on reviews or meta-analyses.

Studies that utilized data from Gene Expression Omnibus datasets.

Review articles, meta-analyses, and case reports.

Studies have not focused on the fibulin family or GC.

These criteria ensure that only relevant and high-quality studies contribute to our review.

Data collection process

One reviewer independently evaluated the quality of the studies and the risk of bias as recommended by the Cochrane Prognosis Methods Group (Table 1) (22). After the final eight studies were selected, one reviewer thoroughly reviewed half of them, while another reviewer independently analyzed the other half. The reviewers conducted their evaluations separately. A meticulous review of all published reports, including tables and figures, was carried out to extract the pertinent information. This process involved seven criteria for assessing the risk of bias, which include: random sequence generation, blinding of both participants and staff, blinding of

evaluators, allocation concealment, selective reporting, incomplete outcome data, and other potential biases.

Result

Reviewed articles were published between 2008 and 2023, all studies performed in China. The studies reported a diverse range of gastric tumors, with the number of specimens ranging from 8 to 197 per study. In this systematic review, were included a cumulative total of 853 tumor samples. The gastric cancer cell lines utilized in these investigations included AGS, Kato III, MKN28, MKN45, SNU1, SNU16, NCI-N87, MGC-803, BGC-83, SGC-7901, SGC-790, and HGC27. In addition to human tumor samples, one study examined gastric tumors from mice.

The studies reviewed used various techniques and methods to conduct the studies, including: Western blot analysis, Reverse transcription-polymerase chain reaction (RT-PCR), quantitative real-time RT–PCR, methylation-specific PCR, Flow cytometric analysis, Immunohistochemistry, Cell culture, plasmid construction, plasmid transfection, Immunofluorescence staining, siRNA transfection, tissue microarray.

Four studies provided data on tumor size measured in centimeters. Histopathological grading was assessed in two of the articles. The T stage was detailed in four studies, with the TNM stage also reported in four studies, and the N stage in three studies. Lymph node metastasis was noted in tumor samples from four studies. Follow-up of patients was limited to two studies, with a maximum duration of 75 months. Tumor differentiation was documented in four studies, while only one study examined the presence of Helicobacter pylori in patients. Kaplan-Meier survival curves were reported to assess patient survival in two studies. The principal features of the seven selected studies are outlined in Table 2.

Liang Feng et al. observed decreased expression of FBLN1 in 24 out of 36 GC. FBLN1 mRNA level in non-cancerous stomach tissues (3.82 ± 0.31) was higher than in cancerous tissues (2.05 ± 0.28) ($P<0.001$). FBLN1 gene expression in non-cancerous tissues was one and a half times that of cancerous tissues. Median Overall survival was decreased in cases with low FBLN1 expression compared to patients with high FBLN1 expression (OS 33.25 vs. 53.41 months, respectively, $P<0.001$). Methylation-specific PCR was used to investigate the relationship between gene promoter methylation and FBLN1 expression level. FBLN1 gene promoter methylation was observed in 61.11% of cancer patients and 27.78% of non-cancer samples. Of the 24 GC samples that had decreased FBLN1 expression, gene promoter methylation was observed in 20 of them. They investigated the effect of FBLN1 on the growth of GC cells with CCK8 assay. Two cell lines that had high expression of FBLN1 were proliferated from control cells. Also, the two cell lines that had high expression of FBLN1 formed fewer colonies compared to the control cells. FBLN1 was injected subcutaneously into mice with GC. Tumor growth in mice with high levels of FBLN1 was slowed down compared to the control. After four weeks, the mean volume and weight of the tumor in the groups that received FBLN1 decreased in comparison with the control group. The high expression of FBLN1 did not affect the progress of the cell cycle in any of its phases. The repressive effects of tumor growth with the increase of FBLN1 were related to the impact of fibulin on apoptosis. High expression of FBLN1 increased cleaved caspase-3 and cleaved PARP protein. To assess whether DNA methylation or histone deacetylase (HDAC) inhibitors play a role in FBLN1 downregulation, all six GC cell lines were treated with 5-aza-dC or NaBT, respectively. Treatment with dimethyl sulfoxide was used as a control. 5-aza-dC and NaBT treatments significantly increased FBLN1 transcript expression in all six GC cell lines (23). The reduction of

fibulin-1 expression in this study and the effect of fibulin-1 injection in gastric tumor show that fibulin-1 can be a new therapeutic approach in GC.

Cheng et al. stated that FBLN1 gene expression was downregulated in all 5 cell lines (AGS, Kato III, MKN28, MKN45, and NCI-N87). After treatment with Aza, the FBLN1 expression was increased in all 5 cell lines. In all five cell lines, FBLN1 methylation was observed in the CpG islands around exon 1 of the FBLN1 gene. The expression of FBLN1 was decreased in most tumor samples compared to normal tissue. FBLN1 gene promoter methylation was also observed in most of the tumor samples, but not in tumor peripheral samples. They did not observe a significant relationship between FBLN1 methylation and clinical features. Since the expression of FBLN1 was decreased in GC tissues and samples, it can be concluded that FBLN1 has a tumor suppressor role, which has lost this function in GC (24).

According to the results of Ma et al. in 2019, FBLN2 gene expression in GC tissue was significantly lower than in tumor margin tissue. β-catenin gene expression in GC tissue was significantly higher than tumor margin. FBLN2 protein was observed in the cytoplasm of cells in 73.47% of GC patients ($p<0.001$). The presence of β-catenin was observed in the cytoplasm of 77.55% of patients ($p<0.001$). There was a significant negative correlation between FBLN2 and β-catenin ($r=-0.361$, $p=0.003$). The results showed that the expression of FBLN2 was decreased in AGS and SGC-790 cell lines. With the help of plasmids, they increased the expression of FBLN2 in AGS and SGC-790 cell lines. After increasing the expression of FBLN2, the expression of β-catenin and cyclin D decreased (25). The role of FBLN2 in controlling the cell cycle is reducing the level of cyclin D, which is lost in GC samples. Western blot analysis in Shen et al.'s study revealed that there was an inverse association between the expression of ITGBL1 and FBLN2 in WT and AR-GC cells. Furthermore, they observed that FBLN2 protein level was considerably decreased in ITGBL1-overexpressing AR-AGS cells, whereas FBLN2 expression was significantly increased in ITGBL1-overexpressing AR-MKN45 cells. FBLN2 expression is substantially lower in M1-stage of disease than in M0-stage. There was a negative association between FBLN2 and ITGBL1 expression in GC tissues. They branched the sufferers into four groups based on the expression levels of ITGBL1 and FBLN2: ITGBL1 high FBLN2 high, ITGBL1 highFBLN2 low, ITGBL1 low FBLN2 high, and ITGBL1 low FBLN2 low. The Kaplan-Meier survival indicated that patients in the ITGBL1 high FBLN2 low group had a worse prognosis than the other three groups. Both univariate and multivariate regression models showed that high T stage, high N stage, high AJCC stage, high expression level of ITGBL1, and low expression level of FBLN2 were threat criteria for overall survival in GC patients. These findings firmly suggest that ITGBL1 may inversely regulate FBLN2 in GC. In AR-AGS cells, where the expression of ITGBL1 was increased, the expression of ITGBL1 decreased with the increase of FBLN2 level. Overexpression of FBLN2 increased the level of CL-PARP1 and CL-Caspase9 and the rate of apoptosis (opposite the effects of increasing ITGBL1). By overexpressing FBLN2, some effects of ITGBL1, including migration and invasion ability, were suppressed in AR-AGS cells (26). The results of the study by Wang et al. showed that the expression of FBLN5 GC samples was higher in stage III-IV samples than in stage I-II ($P<0.05$) (27).

According to the results of Lang et al.'s study, FBLN5 is expressed in gastric tumor tissue and para-carcinoma tissues. This protein was observed in the cytoplasm and nucleus of cells. The expression of FBLN5 in the cytoplasm and nucleus of the tumor was significantly higher than that of the para-carcinoma tissue ($P=0.029$ and $P=0.000$, respectively). The expression of FBLN5 in cancer cytoplasm had a positive and significant correlation with the age of patients ($P=0.044$, $r=0.213$). Older patients expressed higher FBLN5 protein in the cytoplasm. Kaplan-Meier analysis

with log-rank statistical test was performed to analyze the relationship between patient survival and FBLN5 expression in the cytoplasm, FBLN5 expression in the nucleus. The results determined that the expression of FBLN5 in the cytoplasm of cancer cells has a negative correlation with the survival time of patients. The survival time of the high cytoplasmic FBLN5 group was significantly shorter than the low expression group (25.0% vs. 55.6%, P=0.027). Survival of patients with high nuclear FBLN5 expression was also associated with poor prognosis, but this difference was not significant (27.8% vs. 54.5%, P=0.141). The expression of FBLN5 in the cytoplasm is an independent prognostic element in patients with GC (P=0.037) (28).

Chen Li et al. observed increased levels of FBLN5 mRNA in GC tissue compared to match normal tumor-adjacent tissues. Also, the increase in FBLN5 mRNA level was higher in cancerous tissue compared to normal non-cancerous tissue. Increased level of FBLN5 is connected with malignant pathological parameters. Increased level of FBLN5 is associated with poor differentiation, lymph node metastasis, and advanced TNM tumor stage. In the MGC-803 cell line that was transfected with FBLN5 shRNA, a decrease in FBLN5 protein was observed. The reduction of FBLN5 in this cell line decreased cell invasion compared to control cells (29). Unlike other fibulins, the level of FBLN5 was increased in GC tissue. It can be concluded that FBLN5 is involved in the formation of GC through different pathways than other members of the fibulin family.

Tao Chen et al. showed that FBLN5 is associated with malignant pathological features such as metastasis in GC. Hypoxia conditions upregulate the expression of the HIF1α transcription factors such as FBLN5, in endothelial cells. The RT-qPCR determined that NDUFS1 (The largest subunit of a mitochondrial complex I) inhibits the mitochondrial reactive oxygen species (mROS)-HIF1α signaling pathway and leads to a decrease in FBLN5 expression ($P < 0.05$). To confirm these results, they used HIF1α siRNA inhibition in N87 cells. High expression of NDUFS1 inhibited FBLN5 expression in MKN45 GC cells in vitro and in vivo in mice ($P < 0.05$), and deletion of NDUFS1 increased FBLN5 expression. The knockdown of HIF1α disabled the NDUFS1 knockdown-induced upregulation of FBLN5 in NDUFS1-interfering N87 GC cells ($P < 0.05$). Inhibition of mROS also inhibited the expression of FBLN5 by reducing HIF-1α ($P < 0.05$) (30).

Discussion

This systematic review aimed to evaluate the alterations in the expression of fibulin family members in GC and their potential clinical implications. The findings of the included observational studies demonstrate a consistent dysregulation of fibulin proteins, particularly FBLN1, FBLN2, and FBLN5, in GC tissues compared to non-cancerous tissues.

FBLN1 was shown to have decreased expression in most tumor samples, with promoter methylation have function in this downregulation. Reduced FBLN1 expression correlated with worse overall survival, suggesting a tumor suppressor role. Experimental data from animal and cell models reinforced this notion, as increased FBLN1 expression led to reduced tumor growth, increased apoptosis, and diminished colony formation. These results showed that FBLN1 has a potential prognostic biomarker and a possible therapeutic target in GC.

Similar to FBLN1, FBLN2 expression was also downregulated in GC tissue and cell lines. FBLN2 showed an inverse correlation with β-catenin and cyclin D expression, indicating its role in regulating cell cycle and Wnt signaling pathways. Furthermore, FBLN2 overexpression promoted apoptosis and inhibited invasive function of GC cells. These findings support the tumor-suppressive role of FBLN2 and suggest that its decreased expression may contribute to gastric tumorigenesis through deregulated signaling cascades and decreased apoptotic capacity.

FBLN5 expression was upregulated in GC tissues and correlated with adverse clinicopathological parameters, including higher TNM stage, poor differentiation, lymph node metastasis, and reduced survival. High levels of FBLN5 were significantly associated with poor prognosis, making it an independent prognostic factor. Moreover, functional experiments revealed that knockdown of FBLN5 led to decreased invasion of cancer cells, further supporting its pro-tumorigenic role. Interestingly, FBLN5 expression appears to be regulated by hypoxia-inducible factor 1α (HIF-1α), linking it to the hypoxia-related molecular pathways in GC.

The results suggest that fibulin family members exhibit divergent roles in GC. FBLN1 and FBLN2 act as tumor suppressors, while FBLN5 may function as an oncogene. These differences underline the complicated biological behavior of FBLNs in GC and highlight their potential as biomarkers for diagnosis and prognosis as well as therapeutic targets. The epigenetic silencing of FBLN1 and FBLN2 via promoter methylation and the hypoxia-driven upregulation of FBLN5 depicted regulatory mechanisms contributing to GC progression.

Conclusion

Together, these findings underscore the complex regulatory mechanisms and varying impact of fibulin family members in GC, offering insights for potential therapeutic targets and prognostic markers.

Table 1. Assessing the quality of studies

Author and year of publication	Study type	Assessment tool	Risk of bias/Final quality
FKL Chan et al. (2008)	Observational studies	ROBINS-I	Moderate
Chen Li et al. (2014)	Observational studies	ROBINS-I	High
Xiaoxiao Wang et al. (2016)	Observational studies	ROBINS-I	High
Liang Feng et al. (2016)	Observational studies	ROBINS-I	High
Xiaohua Leng et al. (2016)	Observational studies	ROBINS-I	Moderate
HONGPING MA et al. (2019)	Observational studies	ROBINS-I	High
Kanger Shen et al. (2023)	Observational studies	ROBINS-I	Moderate
Tao Chen et al. (2023)	Observational studies	ROBINS-I	Moderate

Abbreviation: ROBINS-I, Risk of Bias in Non-randomized Studies - of Interventions.

Table 2. Characteristics of the eligible studies.

Author	FKL Chan (24)	Chen Li (29)	Xiaoxiao Wang (27)	Liang Feng (23)	Xiaohua Leng (28)	HONGPING MA (25)	Kanger Shen (26)	Tao Chen (30)
Country	China	China	China	China	China	China	China	China
Year of publication	2008	2014	2016	2016	2016	2019	2023	2023
Types of studies	Clinical, in vitro	Clinical, in vitro	Clinical, in vitro	Clinical, in vitro	Clinical, in vitro	Clinical, in vitro	Clinical, in vitro	Clinical, in vitro
Cancer cell line	AGS, Kato III, MKN28, MKN45, SNU1, SNU16 and NCI-N87	MGC-803	AGS	MKN-28, AGS, BGC-83, MKN-45, SGC-7901, and MGC-803	MGC-803	AGS and SGC-790	AGS, HGC27, MKN28 and MKN45	AGS, HGC-27, KATO3, MKN45, N87, and SNU-1
Total patients	102 GC 10 Normal	56	97	197	90	49	93 GC sample/8 female NSG mice	254
Female/ male	46/56	15/41	27/70	70/127	20/70	23/26	34/59	80/174
Average age	Methylated 65.4±13.4 Unmethylated 67.5±10.5	NR	NR	NR	NR	60 years	NR	NR
Range	NR	35-72 years	35-88 years	≤55 58 >55 139	34-83 years	34-78 years	<60 26 >60 67	<60 47 ≥60 207
Gene	FBLN1	FBLN5	FBLN5	FBLN1	FBLN5	FBLN2	FBLN2	FBLN5
Tumor size (cm)	NR	NR	NR	<4 116 ≥4 81	≤5 39 >5 48 Lost 3	≤3 16 >3 33	NR	<5 129 ≥5 125
Grade	NR	NR	Moderately differentiation 41 Poorly differentiation 56	NR	I 0 II 24 III-IV 66	NR	NR	NR
T Stage	NR	NR	I 17 II 27 III 43 IV 10	I 45 II 40 III 44 IV 68	I 4 II 7 III 61 IV 18	NR	I-II 16 III-IV 77	NR
TNM stage	I 15 II 17 III 30 IV 28	NR	NR	NR	I 7 II 30 III 49 IV 4	I-II 20 III-IV 29	NR	I-II 71 III-IV 183
N stage	NR	NR	NR	0 94 1 28 2 37 3 38	0 23 1 16 2 25 3 26	NR	N0 22 N1-3 71	NR
Lymph node metastasis	NR	NR	59 Patients	NR	67 Patients	30	NR	183
Follow up	NR	NR	NR	range 2–66 months	range 0–75 months	NR	NR	NR
Helicobacter pylori	Positive 32 Negative 30	NR	NR	NR	NR	NR	NR	NR
Tumor differentiation	Poor (or no) 41 Moderate 25 Well 6	NR	NR	Poor 91 Moderate 81 Well 25	NR	Poor (or no) 23 Moderate and High 26	NR	Poor 139 Moderate and Well 115

Abbreviation: NR, Not Reported.

Table 3. Oligonucleotide primers of FBLNs			
Author	Gene	Forward	Reverse
FKL Chan (24)	FBLN1	5′-TGCGAATGCAAGACGG-3′	5′-CGTAGACGTTGGCACA-3′
Chen Li (29)	FBLN5	5′-TCGCTATGGTTACTGCCAGCA-3′	5′-TTGGCAAGACCTTCCATCGTC-3′
Liang Feng (23)	FBLN1	5′-TGCGAATGCAAGACGG-3′	5′-CGTAGACGTTGGCACA-3′
HONGPING MA (25)	FBLN2	5'-GAGATCCCTGAGAGTGGCACTGAGG-3'	3'-GAGAAGGCACTCATCCTGGTCATCG-5'

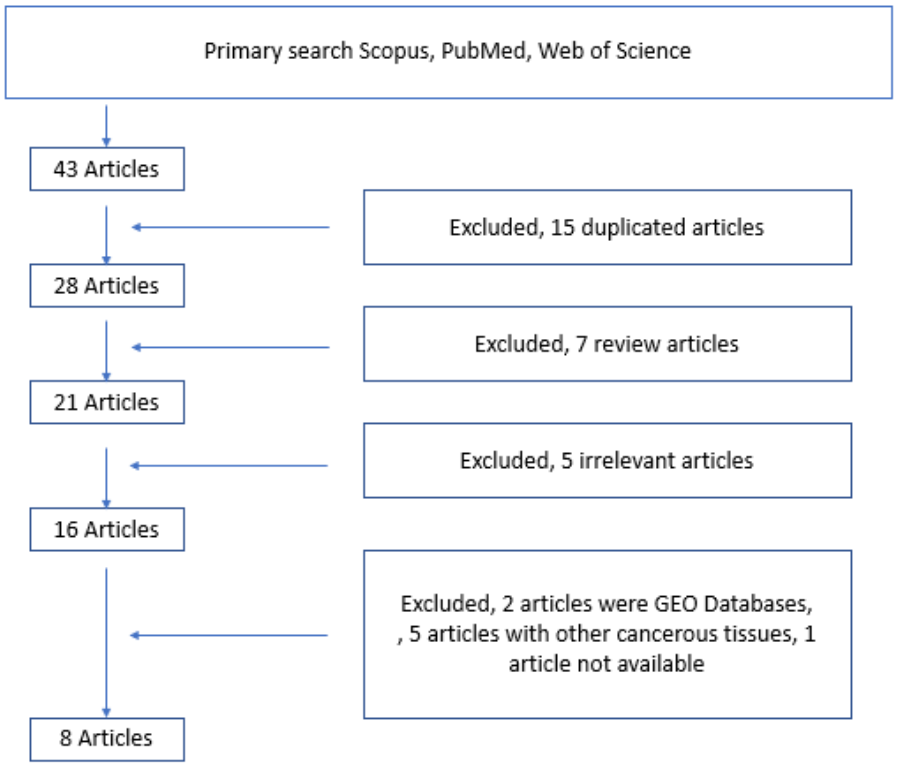

Figure 1

Acknowledgments

The data in this paper are extracted from a research project that was supported by Golestan University of Medical Sciences under grant number 114442.

Funding source

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Ethics statement

Not applicable.

Conflict of interest

The authors declare that they have no competing interests.

Authors' contributions

Jafari designed the research and edited the manuscript. The search in databases was done by Jafari and Mahdizadeh. The manuscript was written by Jafari and Mahdizadeh. All authors read and approved the final manuscript.

Consent for publication

Not applicable.

References

1. Nie W, Hu L, Yan Z, Wang Y, Shi Q, He S, et al. A potential therapeutic approach for gastric cancer: inhibition of LACTB transcript 1. Aging (Albany NY). 2023;15(24):15213-27.
2. Xuan J, Li F, Li J, Gong C, Li J, Mo Z, et al. The role of senescence genes in the treatment, prognosis, and tumor microenvironment of gastric cancer. Am J Transl Res. 2023;15(12):6926-38.
3. Liu Z, Yang M, Shu H, Zhou J. A novel prognostic and therapeutic target biomarker based on complement-related gene signature in gastric cancer. Transl Cancer Res. 2023;12(12):3565-80.
4. Kawada J, Saito T, Kurokawa Y, Kawabata R, Takeno A, Takeoka T, et al. Serum NY-ESO-1 and p53 antibodies as useful tumor markers in gastric cancer. Ann Gastroenterol Surg. 2024;8(2):243-50.
5. Wroblewski LE, Peek RM, Jr. Clinical Pathogenesis, Molecular Mechanisms of Gastric Cancer Development. Curr Top Microbiol Immunol. 2023;444:25-52.
6. Guo M, Hu P, Xie J, Tang K, Hu S, Sun J, et al. Remodeling the immune microenvironment for gastric cancer therapy through antagonism of prostaglandin E2 receptor 4. Genes Dis. 2024;11(4):101164.
7. Feng L, Yao C, Li P, Feng Y, Wang F, Liu YF, et al. Low expression of fibulin-1 correlates with unfavorable prognosis in gastric cancer. Tumour Biol. 2016;37(7):9399-410.
8. Gong J, Jie Y, Xiao C, Zhou W, Li X, Chen Y, et al. Increased Expression of Fibulin-1 Is Associated With Hepatocellular Carcinoma Progression by Regulating the Notch Signaling Pathway. Front Cell Dev Biol. 2020;8:478.
9. Li R, Wu H, Jiang H, Wang Q, Dou Z, Ma H, et al. FBLN5 is targeted by microRNA-27a-3p and suppresses tumorigenesis and progression in high-grade serous ovarian carcinoma. Oncol Rep. 2020;44(5):2143-51.
10. Li WM, Chan TC, Huang SK, Wu WJ, Ke HL, Liang PI, et al. Prognostic Utility of FBLN2 Expression in Patients With Urothelial Carcinoma. Front Oncol. 2020;10:570340.
11. Hang Pham LB, Yoo YR, Park SH, Back SA, Kim SW, Bjørge I, et al. Investigating the effect of fibulin-1 on the differentiation of human nasal inferior turbinate-derived mesenchymal stem cells into osteoblasts. J Biomed Mater Res A. 2017;105(8):2291-8.
12. Sang Y, Mao KM, Huang Y, Wu XF, Wang XF, Ruan L, et al. Relationship between the Plasma Fibulin-1 Levels, Pulse Wave Velocity, and Vascular Age in Asymptomatic Hyperuricemia. Curr Med Sci. 2021;41(1):94-9.
13. Scarinci E, Tropea A, Russo G, Notaristefano G, Messana C, Alesiani O, et al. Increased fibulin-1 plasma levels in polycystic ovary syndrome (PCOS) patients: possible contribution to the link between PCOS and cardiovascular risk. J Endocrinol Invest. 2019;42(1):91-6.
14. Watany MM, Elmashad NM, Badawi R, Hawash N. Serum FBLN1 and STK31 as biomarkers of colorectal cancer and their ability to noninvasively differentiate colorectal cancer from benign polyps. Clin Chim Acta. 2018;483:151-5.

15. Jie Y, Gong J, Xiao C, Zheng J, Zhang Z, Li X, et al. The clinical value of Fibulin-1 for prognosis and its prospective mechanism in intrahepatic cholangiocarcinoma. HPB (Oxford). 2019;21(4):499-507.
16. Ustunyurt E, Dundar B, Simsek D, Temur M. Act of fibulin-1 in preeclamptic patients: can it be a predictive marker? J Matern Fetal Neonatal Med. 2021;34(22):3775-81.
17. Gao Y, Chen T, Hong L. Fibulin-1 promotes intimal hyperplasia after venous stent implantation through ACE mediated angiotensin II signaling. Heliyon. 2024;10(7):e27626.
18. Ma Y, Nenkov M, Schröder DC, Abubrig M, Gassler N, Chen Y. Fibulin 2 Is Hypermethylated and Suppresses Tumor Cell Proliferation through Inhibition of Cell Adhesion and Extracellular Matrix Genes in Non-Small Cell Lung Cancer. Int J Mol Sci. 2021;22(21).
19. Ibrahim AM, Sabet S, El-Ghor AA, Kamel N, Anis SE, Morris JS, et al. Fibulin-2 is required for basement membrane integrity of mammary epithelium. Sci Rep. 2018;8(1):14139.
20. Zhang M, Chen F, Feng S, Liu X, Wang Z, Shen N, et al. FBLN5 as One Presumably Prognostic Gene Potentially Modulating Tumor Immune Microenvironment for Renal Clear Cell Carcinoma in Children and Young Adults. Pharmgenomics Pers Med. 2024;17:27-40.
21. Li Y, Yang X, Lu D. Knockdown of ubiquitin-conjugating enzyme E2T (UBE2T) suppresses lung adenocarcinoma progression via targeting fibulin-5 (FBLN5). Bioengineered. 2022;13(5):11867-80.
22. Hayden JA, van der Windt DA, Cartwright JL, Côté P, Bombardier C. Assessing bias in studies of prognostic factors. Ann Intern Med. 2013;158(4):280-6.
23. Feng L, Yao C, Li P, Feng Y, Wang F, Liu YF, et al. Low expression of fibulin-1 correlates with unfavorable prognosis in gastric cancer. Tumor Biology. 2016;37(7):9399-410.
24. Cheng YY, Jin H, Liu X, Siu JMT, Wong YP, Ng EKO, et al. Fibulin 1 is downregulated through promoter hypermethylation in gastric cancer. British Journal of Cancer. 2008;99(12):2083-7.
25. Ma HP, Lian CH, Song YM. Fibulin-2 inhibits development of gastric cancer by downregulating β-catenin. Oncology Letters. 2019;18(3):2799-804.
26. Shen KE, Xia W, Wang K, Li JT, Xu W, Liu HR, et al. ITGBL1 promotes anoikis resistance and metastasis in human gastric cancer via the AKT/FBLN2 axis. Journal of Cellular and Molecular Medicine. 2024;28(4).
27. Wang X, Zhi Q, Liu S, Xue SL, Shen C, Li Y, et al. Identification of specific biomarkers for gastric adenocarcinoma by ITRAQ proteomic approach. Sci Rep. 2016;6:38871.
28. Leng XH, Yang CG, Xiong B, Hou JX. Increased expression of Fibulin-5 in gastric cancer associated with poor prognosis. International Journal of Clinical and Experimental Medicine. 2016;9(10):19725-32.
29. Shi XY, Wang L, Cao CH, Li ZY, Chen J, Li C. Effect of Fibulin-5 on cell proliferation and invasion in human gastric cancer patients. Asian Pacific Journal of Tropical Medicine. 2014;7(10):787-91.
30. Chen T, Li D, Wang Y, Shen X, Dong A, Dong C, et al. Loss of NDUFS1 promotes gastric cancer progression by activating the mitochondrial ROS-HIF1α-FBLN5 signaling pathway. Br J Cancer. 2023;129(8):1261-73.