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Expression of stathmin in asbestos-like fibers-induced mesothelioma: A preliminary report

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Summary. Background. Mesothelioma is strongly associated with exposure to asbestos fibers, however, recent studies have also linked exposure to "naturally occurring asbestos" fibers with this disease. Fluoro-edenite, a silicate mineral found in the southeast of Biancavilla (Sicily, Italy), has been identified as a potential risk factor for mesothelioma. Unfortunately, this cancer often has a poor prognosis, and current diagnostic and prognostic biomarkers are inadequate. Histological subtype, gender, and age at diagnosis are the most significant parameters for mesothelioma. Stathmin, a cytosolic protein that regulates cell growth and migration and is overexpressed in many human malignancies, has not yet been linked to mesothelioma survival or clinical-pathological variables.

Aim. The aim of this study was to investigate the immunohistochemical expression of stathmin in ten mesothelioma tissue samples with available clinical and follow-up data.

Material and Methods. Paraffin-embedded tissue samples from ten mesothelioma patients were processed for immunohistochemical analyses to evaluate stathmin expression.

Results. Our findings suggest that stathmin overexpression is associated with shorter overall survival in patients with mesothelioma. Furthermore, stathmin expression was significantly correlated with the survival time of mesothelioma patients.

Conclusion. Our results suggest that stathmin expression may serve as a potential prognostic biomarker for mesothelioma. This biomarker could be used to promptly identify patients with poor prognosis and to guide clinicians in the selection of treatment options.

Key words: Mesothelioma, Asbestos-like Fibers, Stathmin, Prognostic biomarker, Fluoro-edenite, Immunohistochemistry

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Introduction

Mesothelioma is a cancer caused by exposure to asbestos and has poor outcomes due to its low response rates to treatments and often late diagnosis. The lack of diagnostic and prognostic biomarkers has hindered early diagnosis of mesothelioma (Filetti et al., 2020). Therefore, it is important to identify new biomarkers for early diagnosis and to understand the molecular mechanisms involved in mesothelioma progression. Stathmin (STMN), a cytosolic protein that regulates cell growth and migration through microtubule dynamics, is overexpressed in various human malignancies (Rubin and Atweh, 2004). Recent studies have suggested the involvement of STMN in the pathogenesis of mesothelioma. However, to date, no studies have correlated the expression of STMN with the survival of mesothelioma patients or with the clinical-pathological variables of the patients.

The incidence of mesothelioma is high in populations with occupational exposure to asbestos, as well as those with para-occupational and environmental exposure (Brims, 2021). Furthermore, a population may be exposed to "naturally occurring asbestos" (NOA), which refers to a mineral as a natural component of soils or rocks that can be released as fibers into the air by human activities or natural weathering processes,

thereby posing a risk of human exposure (Carbone et al., 2016; Filetti et al., 2020). Among the NOA, the most well-known fibers are erionite, winchite, magnesio riebeckite, richterite, Libby asbestos, antigorite and fluoro edenite (FE) fibers (Filetti et al., 2020). Several studies have correlated human exposure to these fibers with mesothelioma (Senygit et al., 2004; Soffritti et al., 2004; Cardile et al., 2007; Groppo and Compagnoni, 2007; Kliment et al., 2009; FitzGerald et al., 2010; Döngel et al., 2013; Fitzgerald and Harty, 2014; Baumann et al., 2015; Baur, 2018; Konen et al., 2019; Loreto et al., 2020a,b).

In particular, FE is a silicate mineral belonging to the amphibole family (Ledda et al., 2020) identified in a quarry located in the southeast of Biancavilla (Sicily, Italy) where the stone material has been used in the local building industry for about 50 years (Ledda et al., 2016a). FE fibers have been acknowledged as a mesothelial carcinogen by the International Agency for Research on Cancer (Grosse et al., 2014), however, epidemiological and pathophysiological information is still limited (Filetti et al., 2021, 2022). The findings of some environmental monitoring projects (Famoso et al., 2012; Bruni et al., 2014) suggest that the population of Biancavilla has been exposed to high levels of amphibole fibers until mitigation measures, adopted in 2001, gradually reduced them to about 0.1-0.4ff/l (Bruni et al., 2014).

To date, the most relevant prognostic parameters for mesothelioma are histological subtype, gender, and age at diagnosis (Delgermaa et al., 2011). Numerous studies have searched for specific and sensitive biomarkers for the early diagnosis of mesothelioma. Recently, higher levels of osteopontin and mesothelin have been associated with a worsening of prognosis. Using osteopontin and mesothelin levels as predictors could play an important role in improving the prognostic capacity of mesothelioma (Pass et al., 2016; Chen et al., 2017). Finally, other molecular alterations associated with mesothelioma are related to the alteration of the expression levels of several factors, particularly autophagy-related (ATG) proteins, which are primarily implicated in different functions of the autophagic process (Follo et al., 2016). Among ATG proteins, the most involved in mesothelioma progression are ATG7 and ATG13, whose high expression levels correlate with increased survival, late recurrence, and death from neoplasm (Follo et al., 2016; Rapisarda et al., 2021).

In this context, several studies have already demonstrated how STMN is overexpressed across a broad range of human malignancies (Rana et al., 2008) including breast cancer (Brattsand et al., 2000; Alli et al., 2007), leukemia (Luo et al., 1994), ovarian cancer (Balachandran et al., 2003), head and neck cancer (Kouzu et al., 2006), prostate cancer (Mistry et al., 2005), and mesothelioma (Zhang et al., 2006; Kim et al., 2007). In particular, in breast cancer, high STMN expression has been associated with a more aggressive phenotype and poor prognosis. Similarly, in ovarian cancer, STMN has been shown to promote tumor growth and metastasis. In non-small cell lung cancer, STMN overexpression has been associated with resistance to chemotherapy. However, these studies did not address the role of STMN in the pathogenesis of mesothelioma (Kim et al., 2007). Some authors found that STMN was highly expressed in mesothelioma and acted as a target of miR-223 that conversely was downregulated in this tumor (Kim et al., 2007; Birnie et al., 2017). To validate these findings, they showed that STMN expression and mesothelioma cell migration were both reduced if normal miR-223 expression was re-established on mesothelioma cell lines (Kim et al., 2007); it has been supposed that an aberrant activation of the c-JUN N-terminal kinase (JNK) pathway could determine high STMN expression in this tumor (Kim et al., 2007; Birnie et al., 2017).

Understanding the role of STMN in tumor development and progression may provide insights into potential therapeutic targets for cancer treatment. Several STMN inhibitors have been developed and are currently being evaluated in preclinical and clinical studies. For instance, a study by Lee et al. (2022) showed that an STMN inhibitor called ABT-737 enhanced the efficacy of radiotherapy in non-small cell lung cancer cells.

Based on the literature background and all the above-mentioned considerations, this study aimed to investigate the relationship between STMN expression, mesothelioma prognosis, and clinical-pathological variables of mesothelioma patients. Understanding the role of STMN in mesothelioma may have important implications for developing new therapeutic strategies and improving our prognostic capacity for mesothelioma.

Materials and methods

Sampling

Since this was a non-interventional retrospective study, neither informed consent nor authorization by the ethics committee were requested and the research complied with the Helsinki Declaration. Mesothelioma samples from ten patients exposed to FE fibers were retrospectively selected. Formalin-fixed and paraffin-embedded (FFPE) tissue samples were obtained from the biobank of the Section of Anatomic Pathology, Department Gian Filippo Ingrassia, University of Catania (Italy). The samples were collected for diagnostic purposes. In agreement with the World Health Organization (WHO) criteria, six cases were histologically classified as epithelioid, three were classified as biphasic subtypes, and one was classified as sarcomatoid (Galateau-Salle et al., 2016). Clinico-pathological and follow-up data were available for all samples under examination. All patient information was transmitted by the National Registry of Mesothelioma (ReNaM). The selected ten patients affected by mesothelioma had been exposed to FE fibers and were

residents in the town of Biancavilla or nearby areas where there was environmental contamination of the silicate fiber.

The cohort of controls was made up of eight patients who did not live in Biancavilla. Furthermore, they did not show neoplastic diseases. These control pleural tissues were collected during surgery for pulmonary emphysema or pleurisy.

Histopathology

After washing in phosphate-buffered saline (PBS; Sigma, Milan, Italy), samples were fixed in 10% buffered formalin (Broggi et al., 2021a) then dehydrated in graded ethyl alcohol, passed in xylene, and finally fixed in paraffin (Loreto et al., 2020a,b). Paraffin-blocks were cut by a microtome into 4-5 μm thick slices and lastly these slices were mounted on silane-coated slides (Dako, Glostrup, Denmark). For the morphological evaluation, sections were stained with Hematoxylin and Eosin and were observed using a Zeiss Axioplan light microscope (Carl Zeiss, Oberkochen, Germany).

Immunohistochemistry

Histologic samples were processed, as previously described (Loreto et al., 2019). After being dewaxed in xylene, rehydrated with graded ethyl alcohol, samples were incubated in 0.3% hydrogen peroxide/methyl alcohol solution for 30 min and finally washed with PBS for antigen retrieval. Samples were put in a microwave oven (750 W) (5 min x 3 cycles) in capped polypropylene slide-holders with citrate buffer (10 mM citric acid, 0.05% Tween 20, pH 6.0; Bio-Optica, Milan, Italy). After dewaxing, samples were incubated at 4°C overnight with anti-stathmin rabbit polyclonal antibody (UniProt ID: P16949; Cell Signaling Technology, Massachusetts, USA) diluted 1:50 in PBS.

Immune complexes treated with a biotinylated link antibody were detected with peroxidase-labeled streptavidin (LSAB + System-HRP, K0690; Dako, Glostrup, Denmark), incubated at room temperature for 10 min. The immunoreaction was visualized by 3,3'-diaminobenzidine and 0.02% hydrogen peroxide solution (DAB substrate Chromogen System; Dako). Samples were counterstained with Mayer's Hematoxylin (Histolab Products AB, Goteborg, Sweden) and mounted in GVA (Zymed Laboratories, San Francisco, CA, USA). The slides thus prepared were evaluated through an Axioplan Zeiss light microscope (Carl Zeiss, Oberkochen, Germany) and the digital images were taken with a Zeiss AxioCam MRc5 digital camera (Carl Zeiss).

STMN immunoexpression was considered positive when brown chromogen was observed at the cytoplasm level. Sections with known antigenic positivity were used as positive controls. Negative sections were obtained from slides treated with PBS without the primary antibodies. The immunohistochemical slides were semi-quantitatively evaluated by two pathologists (G.B. and R.C.) as previously detailed (Broggi et al., 2021b). The Intensity of Staining (IS) was subclassified into four levels (0-3): absence of staining=0, mild staining=1, moderate staining=2, and strong staining=3. Similarly, five levels of Extent Score (ES), the percentage of immunoreactive cells, were identified: <5% (0), 5-30% (1), 31-50% (2), 51-75% (3), and >75% (4). ES was evaluated at magnification 40x. The Immunoreactivity Score (IRS) was obtained multiplying the IS and the ES, ranging from 0 to 12: if the IRS was ≤ the median value of 6, the STMN immunohistochemical expression was considered "low" (L-IRS), while if the IRS was >6, it was considered "high" (H-IRS). These parameters were analyzed by image acquisition software (Axio Vision Release 4.8.2 - SP2 Software, Carl Zeiss Microscopy GmbH, Jena, Germany). The digital images were acquired with a Zeiss AxioCam MRc5 digital camera (Carl Zeiss). The results are presented as mean ± standard deviation (SD).

Statistical analysis

The data were plotted using Prism for Windows v 7.00 (Graphpad Software; CA, USA). Considering the Intensity Reactivity Score (IRS) of the STMN expression values, the Hazard Ratio (HR) was calculated using the Mantel-Haenszel test. Cancer-specific survival analysis was performed using the Kaplan-Meier method, and the Gehan-Breslow-Wilcoxon test was used to compare the survival curves. To evaluate the correlation between clinico-pathological and immunohistochemical data the Spearman correlation was used. In particular, age, gender, pathological subtype, and survival time were correlated with the STMN IRS level. P-values less than 0.05 ($p<0.05$) were considered statistically significant.

Results

Six men and four women made up the cohort of FE-induced mesothelioma cases, and the average age was 68.4±13.9 years (age range: 50-93 years). Eight men made up the cohort of control cases, and the average age was 44±25.5 years (age range: 15-76 years). The clinico-pathological and immunohistochemical information of mesothelioma cases are reported in Table 1.

STMN was detected with a high immunoexpression (IRS>6), as shown in Figure 1A,B, in 50% (n=5) of mesothelioma FE-induced cases (n=2 epithelioid, n=2 biphasic, n=1 sarcomatoid subtypes), while 50% (n=5) of cases (n=4 epithelioid, n=1 biphasic subtypes) showed low immunostaining (IRS≤6, Fig. 1C,D). Immunohistochemical sections of non-neoplastic mesothelial control tissue showed no staining with STMN (Fig. 1E).

Considering the median OS between high and low STMN expression, the Kaplan-Meier method showed a statistically significant association between STMN

expression and increased OS (p=0.0382), and the hazard ratio (HR) was 0.226 with a 95% confidence interval (CI) (0.04901 to 1.042) as shown in Figure 2.

A trend of shorter OS was found in FE-induced mesothelioma patients with STMN overexpression. Of the cases that exhibited a low immunoexpression of STMN 50% showed better prognoses. In particular, a statistically significant correlation of STMN low expression with increased survival was observed with a mean OS of 18 months vs. a mean OS of only 7.5 months for patients with STMN overexpression. Furthermore, there was a significant correlation between STMN expression and the survival time of mesothelioma cases (p=0.0087), shown in Figure 3. No significant

Table 1. The clinico-pathological and immunohistochemical information of mesothelioma cases.

Case	Age (Years)	Gender	Pathological Subtype	Survival Time (Months)	Stathmin IS	Stathmin ES	Stathmin IRS
1	69	M	Epithelioid	1.5	3	3	9
2	50	M	Biphasic (20% Epithelioid, 80% Sarcomatoid)	16	2	4	8
3	69	F	Sarcomatoid	5	2	4	8
4	74	F	Epithelioid	13	2	2	4
5	85	M	Epithelioid	23	2	2	4
6	93	F	Biphasic (40% Epithelioid, 60% Sarcomatoid)	7.5	3	3	9
7	58	F	Epithelioid	18	2	2	4
8	55	M	Epithelioid	37	2	2	4
9	75	M	Biphasic (40% Epithelioid, 60% Sarcomatoid)	60	2	2	4
10	56	M	Epithelioid	12	3	4	9

IS, Intensity of staining; ES, Extent score; IRS, Immunoreactivity score.

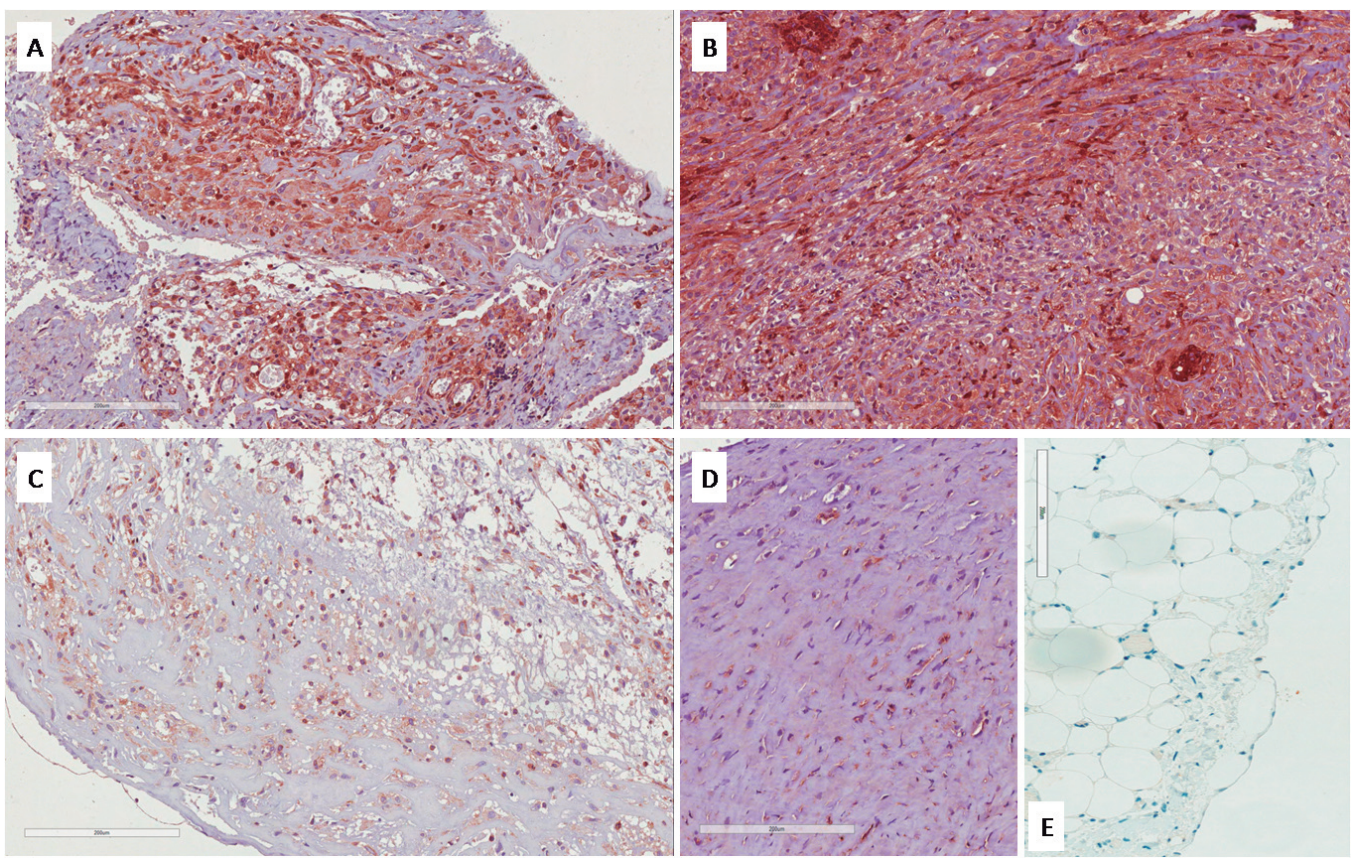

Fig. 1. A. A case of epithelioid cell mesothelioma exhibiting high STMN immunoexpression (IRS >6). **B.** Strong and diffuse STMN immunoexpression (IRS >6) in a biphasic mesothelioma case. **C.** Low expression (IRS ≤6) of STMN in an epithelioid cell mesothelioma. **D.** Low immunoexpression (IRS ≤6) of STMN in mesothelioma exhibiting biphasic morphology. **E.** No immunoreactivity for STMN is seen in normal unaffected mesothelium. Scale bars: 200 μm.

relationship between STMN expression and other clinico-pathological variables (age, sex and mesothelioma pathological subtype) was observed.

Discussion

The results of the current study have shown a statistically significant correlation of low STMN expression with increased patient survival, with a mean overall survival (OS) of 18 months compared to 7.5 months in patients with STMN overexpression, confirming that high STMN expression correlates with poor prognosis. Previous studies demonstrated that STMN is overexpressed in mesothelioma, both at genetic and protein levels (Kim et al., 2007), but no studies, to our knowledge, have been carried out on patients with mesothelioma induced by exposure to FE fibers. A significant correlation between STMN immunoexpression and the survival time of mesothelioma cases has been shown, confirming that high expression of STMN correlates with poorer prognosis. Conversely, no STMN immunoreactivity was observed in non-neoplastic samples of patients without FE exposure. These data confirmed our previous *in silico* evaluation that STMN activation plays a relevant role in malignant transformation and survival rate (Rapisarda et al., 2015). It is worth noting that STMN overexpression in patients with malignant tissue experienced a lower 5-year survival rate than those without (p=0.0007) (Rana et al., 2008). Moreover, Rapisarda et al. already demonstrated that transcriptional levels of STMN were significantly higher in the group of lung cancer and mesothelioma derived from patients exposed to asbestos vs. the other group not exposed to asbestos (Rapisarda et al., 2015).

STMN, also known as oncoprotein 18, has been demonstrated to be upregulated in several cancers such as breast, lung, hepatocellular, and prostate cancers and seems to regulate cell-growth, proliferation, apoptosis, and to mediate cell migration participating in the onset of metastasis; in neoplasms high levels of expression have been associated with larger tumor size, grade and stage (Kim et al., 2007; Belletti and Baldassarre, 2011). STMN expression shows elevated levels in poorly differentiated lung adenocarcinoma vs. moderately and well differentiated tumor cells (Chen et al., 2003). However, in the present paper we did not reveal any relationship between STMN expression and other clinico-pathological variables (age, sex and mesothelioma pathological subtype).

STMN is an eminent cancer-associated gene and a potential target for cancer diagnosis and treatment (Suzuki et al., 2017). The tumor suppressor proteins p53 and p27 are genes that specifically correlated with STMN mRNA (Watanabe et al., 2014). STMN overexpression is associated with the loss of p53 function and a low level of p27, determining an aggressive phenotype of tumor cells (Baldassare et al., 2005; Suzuki et al., 2017). In mesothelioma, the oncogenic role of STMN may be implicated in a specific pathway, such as the low expression of miR-223, which is a tumor suppressor that regulates STMN. High levels of STMN caused by aberrant JNK signaling may suggest a potential tumor-suppressive role for the JNK-miR-233-STMN1 axis (Birnie et al., 2015).

At the protein level, STMN contributes to fetal trophoblast migration and invasion by regulating MMP-2 and MMP-9 expression during pregnancy. This may explain the metalloproteinases overexpression observed in fixed lung tissues of an *in vivo* sheep model with FE fiber exposure (Martinez et al., 2006). Our previous studies already demonstrated the pulmonary and mesothelial inflammatory reaction, triggered by chronic FE exposure, and the activation of the cascade that leads to cellular proliferation and apoptosis disorder (Cardile et al., 2007; Loreto et al., 2008, 2020a,b; Ledda et al., 2016b,c). All these events may switch to cancer onset,

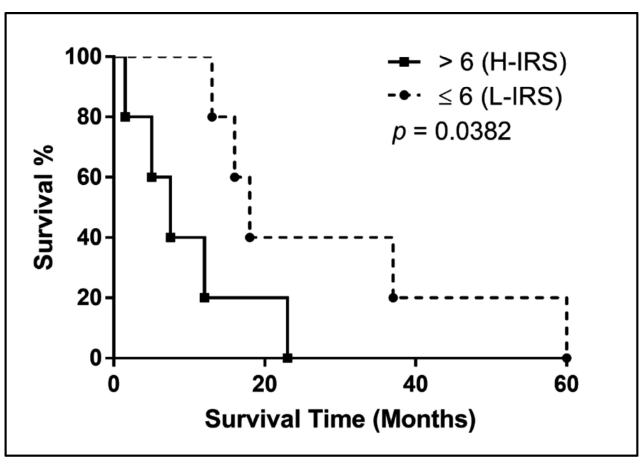

Fig. 2. Median OS between high and low STMN expression. Kaplan-Meier method.

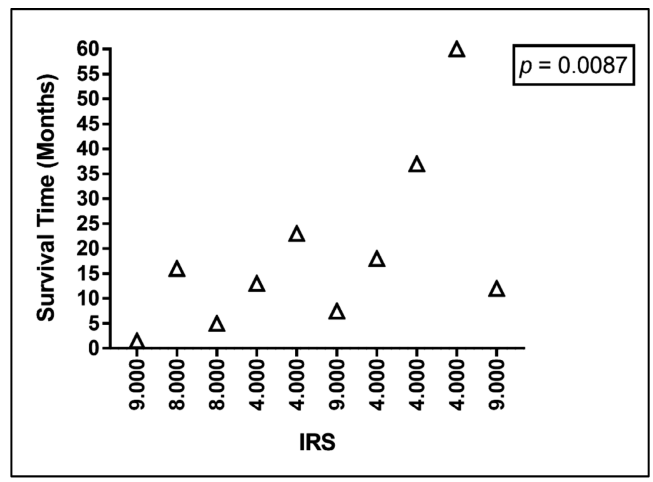

Fig. 3. Spearman correlation between survival time (months) of mesothelioma patients and STMN immunoexpression (IRS).

and STMN overexpression may contribute to the malignant transformation of pleural tissues after FE exposure. Moreover, it has to be considered that another molecular mechanism associated with cancer onset is represented by the epithelial-to-mesenchymal transition (EMT), which is induced mainly by oxidative stress. EMT is associated with asbestos-related diseases and influences the mesothelioma tumor micro-environment (Ramundo et al., 2021). During tumor progression, EMT facilitates cell motility, invasiveness, apoptosis resistance and extracellular matrix production (Dongre and Weinberg, 2018; Katsuno and Derynck, 2021). Reactive oxygen species (ROS) mediate an oxidative stress status responsible for promoting EMT through a mechanism involving GSK-3ß, which in turn regulates SNAI1, NF-kB, ß-catenin and E-cadherin (Cannito et al., 2010).

Lu et al. postulated that STMN, destabilizing microtubule dynamics, promotes the malignant potential in cancer cells through EMT, as microtubule disruption leads to the break-down of the basement membrane, which is the first step of the EMT process (Nakaya et al., 2008; Lu et al., 2014). EMT alteration contributes to fibrosis, neoplasia and cancer onset (Kang and Massagué, 2004; Lopez-Novoa and Nieto, 2009). To better understand how microtubule dynamics participate in oncogenic EMT, it is important to consider that STMN plays a significant role in regulating cellular functions through its microtubule-destabilizing activity. Microtubules are the main component of the cytoskeleton of eukaryotic cells and are involved in essential cellular processes such as cell division, motility, and intracellular transport (Vasiliev and Samoylov, 2013).

The role of STMN is particularly important in therapeutic protocols, as several anti-cancer chemotherapeutics act as microtubule stabilizers or destabilizers, resulting in mitotic arrest and apoptosis as well as limiting cell proliferation (Matesanz et al., 2011; Cortes and Vidal, 2012). STMN is a promising target for more effective therapeutic interventions (Dong et al., 2012; Nemunaitis, 2012) and for personalized medicine.

Biaoxue et al. investigated STMN levels in serum as a potential biomarker for early diagnosis and prognosis for lung adenocarcinoma, demonstrating the feasibility of this tool (Biaoxue et al., 2017). To further explore the potential of STMN as a biomarker, it would be useful to evaluate serum STMN in a Biancavilla cohort of mesothelioma patients exposed to FE, as blood is an easily accessible sample.

However, the weaknesses of this preliminary study include the small sample size due to the difficulties in recruiting patients exposed to FE, as the population in contact with this fiber is relatively small. A larger size would allow for greater statistical significance between STMN expression and other clinico-pathological variables.

In conclusion, immunohistochemical expression of STMN may represent a potential prognostic biomarker for mesothelioma derived from patients exposed to FE fibers, and could serve to promptly provide a better patient prognosis and guide clinicians in choosing therapeutic approaches. Further biomolecular studies on *in vitro* models and on fluid biopsies will help to elucidate the role of STMN as a biomarker and deepen our understanding of the pathogenesis of mesothelioma, potentially leading to new therapeutic approaches.

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References

Alli E., Yang J.M. and Hait W. (2007). Silencing of stathmin induces tumor-suppressor function in breast cancer cell lines harboring mutant p53. Oncogene 26, 1003-1012.

Balachandran R., Welsh M. and Day B. (2003). Altered levels and regulation of stathmin in paclitaxel-resistant ovarian cancer cells. Oncogene 22, 8924-8930.

Baldassarre G., Belletti B., Nicoloso M.S., Schiappacassi M., Vecchione A., Spessotto P., Morrione A., Canzonieri V. and Colombatti A. (2005). p27(Kip1)-stathmin interaction influences sarcoma cell migration and invasion. Cancer Cell 7, 51-63.

Baumann F., Buck B.J., Metcalf R.V., McLaurin B.T., Merkler D.J. and Carbone M. (2015). The presence of asbestos in the natural environment is likely related to mesothelioma in young individuals and women from southern Nevada. J. Thorac. Oncol. 10, 731-737.

Baur X. (2018). Review on the adverse health effects of asbestiform antigorite, a non regulated asbestiform serpentine mineral. Am. J. Ind. Med. 61, 625-630.

Belletti B. and Baldassarre G. (2011). Stathmin: a protein with many tasks. New biomarker and potential target cancer. Expert. Opin. Ther. Targets 15, 1249-1246.

Biaoxue R., Hua L., Tian F. and Wenlong G. (2017). Increased stathmin in serum as a potential tumor marker for lung adenocarcinoma. Jpn. J. Clin. Oncol. 47, 342-349.

Birnie K.A., Yip Y.Y., Ng D.C.H., Kirschner M.B., Reid G., Prele C.M., Musk A.W.B., Lee Y.C.G., Thompson P.J., Mutsaers S.E. and Bradian B. (2015). Loss of miR-223 and JNK signaling contribute to elevated stathmin in malignant pleural mesothelioma. Mol. Cancer

Res. 13, 1106-1118.
Birnie K.A., Prêle C.M., Thompson P.J., Badrian B. and Mutsaers S.E. (2017). Targeting microRNA to improve diagnostic and therapeutic approaches for malignant mesothelioma. Oncotarget 8, 78193-78207.
Brattsand G. (2000). Correlation of oncoprotein 18/stathmin expression in human breast cancer with established prognostic factors. Br. J. Cancer 83, 311-318.
Brims F. (2021). Epidemiology and clinical aspects of malignant pleural mesothelioma. Cancers (Basel) 13, 4194.
Broggi G., Angelico G., Filetti V., Ledda C., Lombardo C., Vitale E., Rapisarda V., Loreto C. and Caltabiano R. (2021a). Immunohistochemical expression of serine and arginine-rich splicing factor 1 (SRSF1) in fluoro-edenite-induced malignant mesothelioma: A preliminary study. Int. J. Environ. Res. Public Health 18, 6249.
Broggi G., Lo Giudice A., Di Mauro M., Asmundo M.G., Pricoco E., Piombino E., Caltabiano R., Morgia G. and Russo G.I. (2021b). SRSF-1 and microvessel density immunohistochemical analysis by semi-automated tissue microarray in prostate cancer patients with diabetes (DIAMOND study). Prostate 81, 882-892.
Bruni B.M., Soggiu M.E., Marsili G., Brancato A., Inglessis M., Palumbo L., Piccardi A., Beccaloni E., Falleni F., Mazziotti Tagliani S. and Pacella A. (2014). Environmental concentrations of fibers with fluoro-edenitic composition and population exposure in Biancavilla (Sicily, Italy). Ann. Ist. Super. Sanita, 50, 119-126.
Cannito S., Novo E., Di Bonzo L.V., Busletta C., Colombatto S. and Parola M. (2010). Epithelial-mesenchymal transition: from molecular mechanisms, redox regulation to implications in human health and disease. Antioxid. Redox. Signal 12, 1383-1430.
Carbone M., Kanodia S., Chao A., Miller A., Wali A., Weissman D., Adjei A., Baumann F., Boffetta P., Buck B., De Perrot M., Dogan U., Gavett S., Gualtieri A., Hassan R., Hesdorffer M., Hirsch F.R., Larson D., Mao W., Masten S. and Malik S. (2016). Consensus report of the 2015 weinman international conference on mesothelioma. J. Thorac. Oncol. 11, 1246-1262.
Cardile V., Lombardo L., Belluso E., Panico A., Capella S. and Balazy M. (2007). Toxicity and carcinogenicity mechanisms of fibrous antigorite. Int. J. Environ. Res. Public Health 4, 1-9.
Chen G., Wang H., Gharib T.G., Huang C.C., Thomas D.G., Shedden K.A. and Beer D.G. (2003). Overexpression of oncoprotein 18 correlates with poor differentiation in lung adenocarcinomas. Mol. Cell. Proteomics 2, 107-116.
Chen Z., Gaudino G., Pass H.I., Carbone M. and Yang H. (2017). Diagnostic and prognostic biomarkers for malignant mesothelioma: an update. Transl. Lung Cancer Res. 6, 259-269.
Cortes J. and Vidal M. (2012). Beyond taxanes: the next generation of microtubule-targeting agents. Breast Cancer Res. Treat. 133, 821-830.
Delgermaa V., Takahashi K., Park E.K., Le G.V., Hara T. and Sorahan T. (2011). Global mesothelioma deaths reported to the World Health Organization between 1994 and 2008. Bull. World Health Organ 89, 716-724.
Dong B., Mu L., Qin X., Qiao W., Liu X., Yang L., Xue L., Rainov N.G. and Liu X. (2012). Stathmin expression in glioma-derived microvascular endothelial cells: A novel therapeutic target. Oncol. Rep. 27, 714-718.
Döngel I., Bayram M., Bkan N.D., Yalçın H. and Gültürk S. (2013). Is living close to ophiolites related to asbestos related diseases? Cross sectional study. Respir. Med. 107, 870-874.
Dongre A. and Weinberg R.A. (2018). New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer. Nat. Rev. Mol. Cell. Biol. 20, 69-84.
Famoso D., Mangiameli M., Roccaro P., Musumeci G. and Vagliasindi F.G.A. (2012). Asbestiform fibers in the Biancavilla site of national interest (Sicily, Italy): review of environmental data via GIS platforms. Rev. Environ. Sci. Biotechnol. 11, 417-427.
Filetti V., Vitale E., Broggi G., Hagnäs M.P., Candido S., Spina A. and Lombardo C. (2020). Update of in vitro, in vivo and ex vivo fluoro-edenite effects on malignant mesothelioma: A systematic review (Review). Biomed. Rep. 13, 60.
Filetti V., Loreto C., Falzone L., Lombardo C., Cannizzaro E., Castorina S., Ledda C. and Rapisarda V. (2021). Diagnostic and prognostic value of three microRNAs in environmental asbestiform fibers-associated malignant mesothelioma. J. Pers. Med. 11, 1205.
Filetti V., La Ferlita A., Di Maria A., Cardile V., Graziano A.C.E., Rapisarda V., Ledda C., Pulvirenti A. and Loreto C. (2022). Dysregulation of microRNAs and tRNA-derived ncRNAs in mesothelial and mesothelioma cell lines after asbestiform fiber exposure. Sci. Rep. 12, 9181.
Fitzgerald S.M. and Harty E.A. (2014). Antigorite: Is it the forgotten asbestos? Prof. Saf. 59, 43 48.
FitzGerald J., Eggleton R. and Keeling J. (2010). Antigorite from Rowland flat, South Australia: asbestiform character. Eur. J. Mineral. 22, 525-533.
Follo C., Barbone D., Richards W.G., Bueno R. and Broaddus C. (2016). Autophagy initiation correlates with the autophagic flux in 3D models of mesothelioma and with patient outcome. Autophagy 12, 1180-1194.
Galateau-Salle F., Churg A., Roggli V. and Travis W.D. (2016). The 2015 World Health Organization classification of tumors of the pleura: advances since the 2004 classification. J. Thorac. Oncol 11, 142-154.
Groppo C. and Compagnoni R. (2007). Ubiquitous fibrous antigorite veins from the Lanzo Ultramafic Massif, Internal Western Alps (Italy): Characterization and genetic conditions. Per. Mineral. 76, 169 181.
Grosse Y., Loomis D., Guyton K.Z., Lauby Secretan B., El Ghissassi F., Bouvard V., Benbrahim Tallaa L., Guha N., Scoccianti C., Mattock H. and Straif K. (2014). Carcinogenicity of fluoro edenite, silicon carbide fibres and whiskers, and carbon nanotubes. Lancet Oncol. 15, 1427 1428.
Kang Y. and Massagué J. (2004). Epithelial-mesenchimal transitions: Twist in development and metastasis. Cell 118, 277-279.
Katsuno Y. and Derynck R. (2021). Epithelial plasticity, epithelial-mesenchymal transition, and the TGF-β family. Dev. Cell 56, 726-746.
Kim J.Y., Harvard C., You L., Xu Z., Kuchenbecker K., Baehner R. and Jablons D. (2007). Stathmin is overexpressed in malignant mesothelioma. Anticancer Res. 27, 39-44.
Kliment C.R., Clemens K. and Oury T.D. (2009). North American erionite associated mesothelioma with pleural plaques and pulmonary fibrosis: a case report. Int. J. Clin. Exp. Pathol. 2, 407-410.
Konen T., Johnson J.E., Lindgren P. and Williams A. (2019), Cancer incidence and mortality associated with non occupational and low dose exposure to Libby vermiculite in Minnesota. Environ. Res. 175, 449-456.
Kouzu Y., Uzawa K., Koike H., Saito K., Nakashima D., Higo M., Endo

Y., Kasamatsu A., Shiiba M., Bukawa H., Yokoe H. and Tanzawa H. (2006). Overexpression of stathmin in oral squamous-cell carcinoma: correlation with tumour progression and poor prognosis. Br. J. Cancer 94, 717-723.

Lee J.M., Kim H.S., Chang Y.S., Lee J.G., Cho J. and Kim E.Y. (2022). ABT-737, a BH3 mimetic, enhances the therapeutic effects of ionizing radiation in K-ras mutant non-small cell lung cancer preclinical model. Yonsei Med. J. 63, 16-25.

Ledda C., Lombardo C., Tendi E.A., Hagnas M., Paravizzini G, Filetti V. and Rapisarda V. (2020). Pathway of inflammation due to asbestiform fiber “fluoro-edenite” exposure: An update. Curr. Resp. Med. Rev. 16, 73-75.

Ledda C., Pomara C., Bracci M., Mangano D., Ricceri V., Musumeci A., Ferrante M., Musumeci G., Loreto C., Fenga C., Santarelli L. and Rapisarda V. (2016a) Natural carcinogenic fiber and pleural plaques assessment in a general population: A cross-sectional study. Environ. Res. 150, 23-29.

Ledda C., Loreto C., Matera S., Massimino N., Cannizzaro E., Musumeci A., Migliore M., Fenga C., Pomara C. and Rapisarda V. (2016b). Early effects of fluoro-edenite: correlation between IL-18 serum levels and pleural and parenchymal abnormalities. Future Oncol. 12, 59-62.

Ledda C., Loreto C., Pomara C., Rapisarda G., Fiore M., Ferrante M., Bracci M., Santarelli L., Fenga C. and Rapisarda V. (2016c). Sheep lymph-nodes as a biological indicator of environmental exposure to fluoro-edenite. Environ. Res. 147, 97-101.

Lopez-Novoa J.M. and Nieto M.A. (2009). Inflammation and EMT: an alliance towards organ fibrosis and cancer progression. EMBO Mol. Med. 1, 303-314.

Loreto C., Rapisarda V., Carnazza M.L., Musumeci G., Valentino M., Fenga C and Martinez G. (2008). Fluoro-edenite induce lung cell apoptosis: an in vivo study. Histol. Histopathol. 23, 319-326.

Loreto C., Lombardo C., Caltabiano R., Filetti V., Vitale E., Seminara D., Castorina S., Fenga C., Ledda C. and Rapisarda V. (2019). Immunohistochemical expression and localization of MMP-9, MMP-13, E-Cadherin and Ki-67 in road pavers' skin chronically exposed to bitumen products. Histol. Histopathol. 34, 1141-1150.

Loreto C., Caltabiano R., Graziano A.C.E., Castorina S., Lombardo C., Filetti V, Vitale E., Rapisarda G., Cardile V, Ledda C. and Rapisarda V. (2020a). Defense and protection mechanisms in lung exposed to asbestiform fiber: the role of macrophage migration inhibitory factor and heme oxygenase-1. Eur. J. Histochem. 64, 3073.

Loreto C., Lombardo C., Caltabiano R., Ledda C., Hagnas M., Filetti V. and Rapisarda V. (2020b). An in vivo immunohistochemical study on MacroH2A.1 in lung and lymph-node tissues exposed to an asbestiform fiber. Curr. Mol. Med. 20, 653-660.

Lu Y., Liu C., Xu Y.F., Cheng H., Shi S., WU C.T. and Yu X.J. (2014). Stathmin destabilizing microtubule dynamics promotes malignant potential in cancer cells by epithelial-mesenchymal transition. Hepatobiliary Pancreat. Dis. Int. 13, 386-391.

Luo X.N., Mookerjee B., Ferrari A., Mistry S. and Atweh G.F. (1994). Regulation of phosphoprotein p18 in leukemic cells. Cell cycle regulated phosphorylation by p34cdc2 kinase. J. Biol. Chem. 269, 10312-10318.

Martinez G., Loreto C., Rapisarda V., Musumeci G., Valentino M. and Carnazza M.L. (2006). Effects of exposure to fluoro-edenite fibre pollution on the respiratory system: an in vivo mode. Histol. Histopathol. 21, 595-601.

Matesanz R., Rodriguez-Salarichs J., Pera B., Canales A., Andreu J.M., Jimenez-Barbero J., Bras W., Nales A., Fang W.S. and Diaz J.F. (2011). Modulation of microtubule interprotofilament interactions by modified taxanes. Biophys. J. 101, 2970-2980.

Mistry S.J., Bank A. and Atweh G.F. (2005). Targeting stathmin in prostate cancer. Mol. Cancer Ther. 4, 1821-1829.

Nakaya Y., Sukowati E.W., Wu Y. and Sheng G. (2008). RhoA and microtulule dynamics control cell-basement membrane interaction in EMT during gastrulation. Nat. Cell Biol. 10, 765-775.

Nemunaitis J. (2012). Stathmin 1: a protein with many tasks. New biomarker and potential target in cancer. Expert. Opin. Ther. Targets 16, 631-634.

Pass H.I., Goparaju C., Espin-Garcia O., Donington J., Carbone M., Patel D., Chen Z., Feld R., Cho J., Gadgeel S., Wozniak A., Chachoua A., Leighl N., Tsao M.S., De Parrot M., Xu W. and Liu G. (2016). Plasma biomarker enrichment of clinical prognostic indices in malignant pleural mesothelioma. J. Thorac. Oncol. 11, 900-909.

Ramundo V., Zanirato G. and Aldieri E. (2021). The epithelial-to-mesenchimal transition (EMT) in the development and metastasis of malignant pleural mesothelioma. Int. J. Mol. Sci. 22, 12216.

Rana S., Maples P.B., Senzer N. and Nemunaitis J. (2008). Stathmin 1: a novel therapeutic target for anticancer activity. Expert Rev. Anticancer Ther. 8, 1461-1470.

Rapisarda V., Ledda C., Migliore M., Salemi R., Musumeci A., Bracci M., Marconi A., Loreto C. and Libra M. (2015). FBLN-3 as a biomarker of pleural plaques in workers occupationally exposed to carcinogenic fibers: a pilot study. Future Oncol. 11, 35-37.

Rapisarda V., Broggi G., Caltabiano R., Lombardo C., Castorina S., Trovato A., Ledda C., Filetti V., and Loreto C. (2021). ATG7 immunohistochemical expression in malignant pleural mesothelioma. A preliminary report. Histol. Histopathol. 36, 1301-1308.

Rubin C.I. and Atweh G.F. (2004). The role of stathmin in the regulation of the cell cycle. J. Cell Biochem. 93, 242-250.

Senyigit A., Dalgic A., Kavak O. and Tanrikulu A.C. (2004). Determination of environmental exposure to asbestos (tremolite) and mesothelioma risks in the southeastern region of Turkey. Arch. Environ. Health 59, 658 662.

Soffritti M., Minardi F., Bua L., Degli Esposti D. and Belpoggi F. (2004). First experimental evidence of peritoneal and pleural mesotheliomas induced by FE fibres present in Etnean volcanic material from Biancavilla (Sicily, Italy). Eur. J. Oncol. 9, 169 175.

Suzuki S., Yokobori T., Altan B., Hara K., Ozawa D., Tanaka N., Sakai M., Sano A., Sohda M., Bao H., Fukuchi M., Miyazaki T., Kaira K., Asao T. and Kuwan H. (2017). High stathmin 1 expression is associated with poor prognosis and chemoradiation resistance in esophageal squamous cell carcinoma. Int. J. Oncol. 50, 1184-1190.

Vasiliev J.M. and Samoylov V.I. (2013). Regulatory functions of microtuules. Biochemistry (Mosc) 7, 37-40.

Watanabe A., Suzuki H., Yokobori T., Tsukagoshi M., Altan B., Kubo N., Suzuki S., Araki K., Wada S., Kashiwabara K., Hosouchi Y. and Kuwano H. (2014). Stathmin1 regulates p27 expression, proliferation and drug resistance, resulting in poor clinical prognosis in cholangiocarcinoma. Cancer Sci. 105, 690-696.

Zhang H.Z., Wang Y., Gao P., Lin F., Liu L., Yu B. and Wang R. (2006). Silencing stathmin gene expression by survivin promoter-driven siRNA vector to reverse malignant phenotype of tumor cells. Cancer Biol. Ther. 5, 1457-1461.

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