# Pleural mesothelioma from fluoro-edenite exposure: PACAP and PAC1 receptor. A preliminary report

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ABSTRACT

Pleural mesothelioma is a devastating malignancy primarily associated with asbestos exposure. However, emerging evidence suggests that exposure to fluoro-edenite fibers, a naturally occurring mineral fiber, can also lead to the development of pleural mesothelioma. In this study, based on the hypothesis that pituitary adenylate cyclase-activating polypeptide (PACAP) and PACAP-preferring receptor (PAC1R) expressions could be dysregulated in pleural mesothelioma samples and that they could potentially act as diagnostic or prognostic biomarkers, we aimed to investigate the immunohistochemical expression of PACAP and PAC1R in pleural biopsies from patients with pleural mesothelioma exposed to fluoro-edenite fibers. A total of 12 patients were included in this study, and their biopsies were processed for immunohistochemical analysis to evaluate the expression of PACAP and its receptor. The study revealed a correlation between the overexpression of PACAP and PAC1R and shorter overall survival in patients with malignant mesothelioma. These findings suggest that PACAP and PAC1R expression levels could serve as potential prognostic biomarkers for malignant mesothelioma. Furthermore, the immunohistochemical analysis of PACAP and PAC1R may provide valuable information for clinicians to guide therapeutic decisions and identify patients with poorer prognosis.

**Key words:** pleural mesothelioma; asbestos-like fibers; PACAP; PAC1R; fluoro-edenite; immunohistochemistry.



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**Conflict of interest:** the authors declare no conflict of interest.

**Ethical approval:** this study is a non-interventional retrospective investigation, ethical consent from the Ethics Committee was not required; the research adhered to the principles outlined in the Helsinki Declaration.

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## Introduction

Pleural mesothelioma is a highly aggressive and often fatal cancer that originates from the pleural lining of the lungs and is primarily associated with exposure to asbestos fibers.[1] Despite efforts to regulate and minimize asbestos exposure, the incidence of mesothelioma remains high, particularly in individuals with occupational or environmental exposure to asbestos-containing materials. However, recent studies have highlighted the role of other mineral fibers, such as fluoro-edenite (FE), in the development of mesothelioma.[2-4]

FE is a fibrous mineral that belongs to the amphibole group, and its presence has been documented in certain geographic regions with volcanic activity, particularly in some areas of Italy.[2-4] Epidemiological investigations have identified an increased incidence of pleural mesothelioma in individuals exposed to FE fibers, suggesting that this mineral fiber poses a significant health risk.[5,6] In 2022, the International Agency for Research on Cancer (IARC) classified FE as carcinogenic to human.[7] Despite the recognition of FE as a potential carcinogen and its non malignat respiratory effects (*i.e.*, pleural plaques, parenchymal abnormalities, pleural effusion), the exact mechanisms by which it induces pleural abnormalities are not fully understood. Literature data suggest that FE fibers trigger an inflammatory organ reaction by the release of chemokines and cytokines, and promote immunological induction of autoimmune disease.[8] In particular by analyzing *in vitro*, *in vivo* and *ex vivo* studies,[9] it has been demonstrated that FE fibers induce cellular multinucleation, increase in cell size, ROS production leading to DNA mutations and enhanced signal transduction that may lead to activation of oncogenes, chronic inflammation, deregulation of the methylation state, aberrant microRNAs expression and hereby increase risk of cancer development.[10,11]

Moreover, damage to epithelial cells and macrophages results in the increased expression of genes regulating the production of peptide growth factors, which facilitate cell proliferation and the excessive formation of connective tissue.[12] Pleural mesothelioma arises when carcinogenic asbestos fibers induce anomalies in specific gene sets responsible for controlling cell division.[13,14] Asbestos induces genetic abnormalities through its binding to chromosomal DNA and the generation of oxygen radicals, thereby facilitating the occurrence of mutations.[3,15-19]

In recent years, there has been growing interest in exploring the role of various signaling molecules and biomarkers in mesothelioma. PACAP, a neuropeptide with diverse physiological functions, has emerged as potential modulators of tumor progression and metastasis.[20,21] It exerts its biological effects through interaction with specific G protein-coupled receptors known as PAC1, VPAC1, and VPAC2 receptors.[22] PAC1R represents the high-affinity and PACAP-selective receptor, whose stimulation can activate different signaling cascades mediated by adenylate-cyclase (AC) or phospholipase-C (PLC) activation as well as calcium-regulated mechanisms.[23,24] Furthermore, some effects of the peptide are mediated by the activation of the activity-dependent neuroprotective protein.[25] Dysregulation of PACAP and PAC1R signaling has been implicated in several types of cancers. In particular the peptide exerts a controversial role as it can inhibit the proliferation and migration of different tumour cells such as glioblastoma cells, prostate cancer cells and neuroblastoma cells[26-30] or promote tumour growth in osteosarcoma cells and human colonic tumour cells.[31,32] Although the expression and functional significance of PACAP and its preferring receptor have been investigated in various cancers, their role in pleural mesothelioma, particularly in the context of FE exposure, remains poorly understood. Understanding the involvement of PACAP and PAC1R in FE-induced mesothelioma could provide valuable insights into the underlying mechanisms of disease development and progression.

The aim of this study was to examine the expression patterns of PACAP and PAC1R in pleural biopsies obtained from patients with pleural mesothelioma exposed to FE fibers. We hypothesized that PACAP and PAC1R may be dysregulated in mesothelioma samples and could potentially serve as diagnostic or prognostic biomarkers. PACAP and PAC1R, with their diverse roles in cellular signaling, have the potential to provide valuable insights into the pathogenesis of mesothelioma and may serve as important diagnostic or prognostic markers and provide a basis for future research exploring targeted therapies.

## Materials and Methods

### Sampling

As this study is a non-interventional retrospective investigation, ethical consent from the ethics committee was not required, and the research adhered to the principles outlined in the Helsinki Declaration. Retrospectively, we selected formalin-fixed and paraffin-embedded (FFPE) tissue samples from twelve patients who had been exposed to FE fibers. These samples were obtained from the biobank of the Section of Anatomic Pathology, Department Gian Filippo Ingrassia, University of Catania (Italy), and were originally collected for diagnostic purposes. The selection of cases was in accordance with the histological classification criteria of the World Health Organization (WHO), with six cases classified as epithelioid, three as biphasic subtypes, and three as sarcomatoid.[33] Clinical-pathological data and follow-up information were available for all samples through the National Registry of Mesothelioma (ReNaM). It is important to note that the ten selected patients with malignant pleural mesothelioma (MPM) and exposure to FE fibers were residents of the town of Biancavilla or neighboring areas affected by environmental contamination with silicate fibers.

The control group comprised eight patients who were not residents of Biancavilla and did not present neoplastic diseases. Control pleural tissues were obtained during surgeries for pulmonary emphysema or pleurisy.

### Histopathology

Following washing with phosphate-buffered saline (PBS; Sigma, Milan, Italy), samples were fixed in 10% buffered formalin.[34] Subsequently, they were dehydrated in graded ethyl alcohol, cleared in xylene, and finally embedded in paraffin. Paraffin blocks were sectioned into 4-5 µm thick slices using a microtome, and these sections were mounted on silane-coated slides (Dako, Glostrup, Denmark). For morphological evaluation, sections were stained with Hematoxylin and Eosin and examined under a Zeiss Axioplan light microscope (Carl Zeiss, Oberkochen, Germany).

### Immunohistochemistry

Histologic samples were processed as previously described.[35] After dewaxing in xylene and rehydration in graded ethyl alcohol, samples were treated with a 0.3% hydrogen peroxide/methanol solution for 30 min, followed by washing with PBS for antigen retrieval. Subsequently, the samples were subjected to antigen retrieval in a microwave oven (750 W) using capped polypropylene slide-holders with citrate buffer (10 mM citric acid, 0.05% Tween 20, pH 6.0; Bio-Optica, Milan, Italy). Following dewaxing, the samples were incubated overnight at 4°C with an anti-PACAP and anti-PAC1 receptor mouse monoclonal antibody (PACAP sc-166180; PAC1 Receptor sc-100315; Santa Cruz Biotechnology, Dallas, TX, USA) diluted 1:50 in PBS.

Immune complexes were detected using a biotinylated link antibody, followed by peroxidase-labeled streptavidin (LSAB + System-HRP, K0690; Dako). The immunoreaction was visualized using 3,3'-diaminobenzidine and 0.02% hydrogen peroxide solution (DAB substrate Chromogen System; Dako). Sections were counterstained with Mayer's Hematoxylin (Histolab Products AB, Goteborg, Sweden) and mounted using GVA (Zymed Laboratories, San Francisco, CA, USA). The prepared slides were evaluated using an Axioplan Zeiss light microscope (Carl Zeiss), and digital images were captured using a Zeiss AxioCam MRc5 digital camera (Carl Zeiss).

Pacap and PAC1R immunostainings were considered positive when the brown chromogen was seen within cell cytoplasms. Human unaffected liver and stomach tissue samples were used as positive external controls. Negative control sections were obtained by omitting the primary antibodies. The morphometric and densitometric counts were performed by randomly observing seven fields, about 600,000 μm$^2$, for each slide. The percentage of cells (morphometric analysis) which were positively stained with anti-Pacap and Pacap Receptor antibodies was assessed as % positive, dark brown pixel$^2$ of the evaluated fields, while the intensity of staining (densitometric analysis) was reported as densitometric count (pixel$^2$) of positive, dark brown pixel$^2$ of the evaluated fields. An image acquisition software (Axio Vision Release 4.8.2 - SP2 Software, Carl Zeiss Microscopy GmbH, Jena, Germany) was used to analyze these parameters and the results were reported as mean ± standard deviation.

## Statistical Analysis

The data were plotted using Prism for Windows v 9.0.0 (Graphpad Software; CA, USA). Data were tested for normality with the D'Agostino and Pearson test. Paired *t*-test and Wilcoxon test were used for comparisons between two means; *p*-values less than 0.05 ($p<0.05$) was considered statistically significant. To compare densitometric and morphometric analyzes according to cancer subtype two-way ANOVA was used.

## Results

Our series included seven men and five women affected by FE-induced MPMs, with an age ranging from 50 to 93 years (average age: 67±12.4 years). Histologically, six tumors were composed of polygonal cells with large-sized, eosinophilic cytoplasm and vesicular nuclei, arranged in nests/cords and were diagnosed as epithelioid MPMs, three cases exhibited a spindled morphology consisting of elongated cells with ovoid nuclei, arranged in short intersecting fascicles and set in a prominent collagenous stroma, and were classified as sarcomatoid subtypes, and the remaining three showed both morphologies and were classified as biphasic, accordingly. Eight cases of unaffected mesothelium were part of the control group, and the average age was 44±25.5 years (age range: 15-76 years). Table 1 summarizes the clinico-pathological and immunohistochemical features of the MPM cases. Figures 1-3 show the differential immunohistochemical expression of PACAP and PAC1 R in control tissue and in epithelioid and sarcomatoid MPMs. In more detail, regardless of the cell type, PAC1R was highly expressed in all cases. Conversely, the immunohistochemical expression of PACAP was high in all cases with epithelioid morphology (6/12 cases, 50%), while it was low or absent in biphasic and sarcomatoid subtypes, respectively. Interestingly, all cases of unaffected mesothelial control tissue showed immunoreactivity for both PAC1R and PACAP. The D'Agostino and Pearson test showed that the percentage areas stained with PACAP (area %) were not normally distributed while the percentage areas stained with PAC1R showed a normal distribution. The level of both PACAP and PAC1R staining intensity of positive areas (pixel$^2$) in cases of MPM and controls showed all variables not normally distributed. In Figure 4, the morphometric analysis of PACAP and PAC1R in MPMs tissue compared to the controls tissue were shown. In particular, Figure 4A showed the significantly increase of morphometric expression of PACAP in MPMs compared to the controls with $p=0.0068$; Figure 4B showed the significantly increase of morphometric expression of PAC1R in MPMs compared to the controls



**Table 1.** Clinico-pathologic and immunohistochemical features of malignant pleural mesotheliomas from our series.

| Case | Age (years) | Gender | Morphology | Survival time (months) | Area % PACAP | Area % PAC1R | Pixel2 PACAP | Pixel2 PAC1R |
|---|---|---|---|---|---|---|---|---|
| 1 | 69 | Male | Epithelioid | 1.5 | 2.2943 | 5.0086 | 28694 | 62641 |
| 2 | 50 | Male | Biphasic (20% epithelioid, 80% sarcomatoid) | 16 | 2.3546 | 4.9765 | 30000 | 60849 |
| 3 | 69 | Female | Sarcomatoid | 5 | 0 | 4.2474 | 0 | 53120 |
| 4 | 74 | Female | Epithelioid | 13 | 2.4567 | 5.2431 | 25693 | 58932 |
| 5 | 85 | Male | Epithelioid | 23 | 1.8538 | 5.4521 | 27921 | 64537 |
| 6 | 93 | Female | Biphasic (40% epithelioid, 60% sarcomatoid) | 7.5 | 2.1876 | 4.2417 | 26547 | 62345 |
| 7 | 58 | Female | Epithelioid | 18 | 1.6753 | 5.0943 | 29012 | 52980 |
| 8 | 55 | Male | Epithelioid | 37 | 2.9875 | 5.0732 | 28590 | 60983 |
| 9 | 75 | Male | Biphasic (40% epithelioid, 60% sarcomatoid) | 60 | 2.0127 | 5.3487 | 27653 | 63621 |
| 10 | 56 | Male | Epithelioid | 12 | 2.0654 | 4.9741 | 28298 | 63641 |
| 11 | 61 | Male | Sarcomatoid | 6.5 | 0 | 4.5000 | 0 | 54000 |
| 12 | 59 | Female | Sarcomatoid | 5 | 0 | 4.3000 | 0 | 53432 |

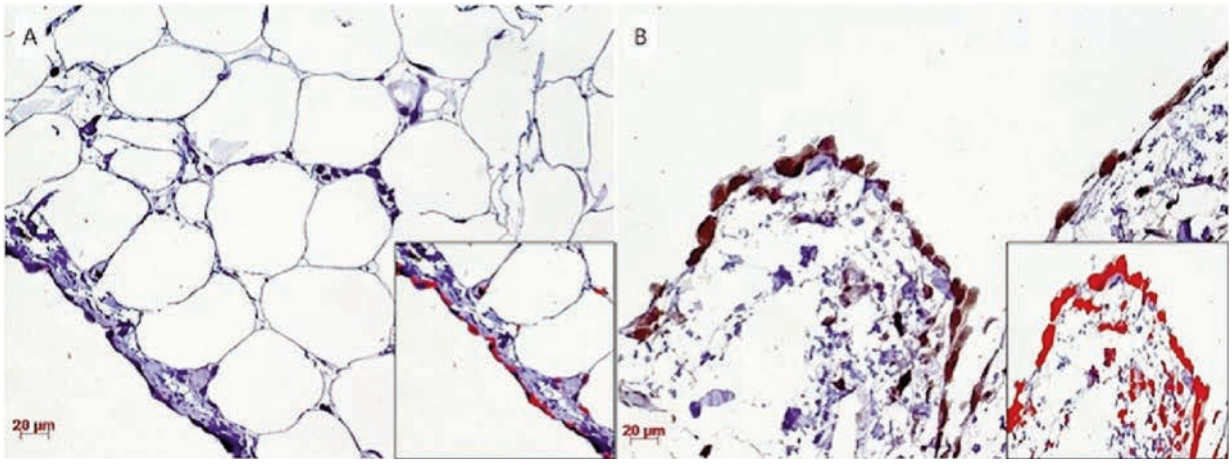


**Figure 1. A**) Immunohistochemical section of unaffected mesothelium showing positive staining with PACAP (immunoperoxidase staining); PACAP immunostaining software image analysis in which mainly a high immunostained area (red color) was detected (insert). **B**) Positive immunostaining with PAC1R in unaffected mesothelial control tissue (immunoperoxidase staining); PAC1R immunostaining software image analysis in which mainly a high immunostained area (red color) was detected (insert).

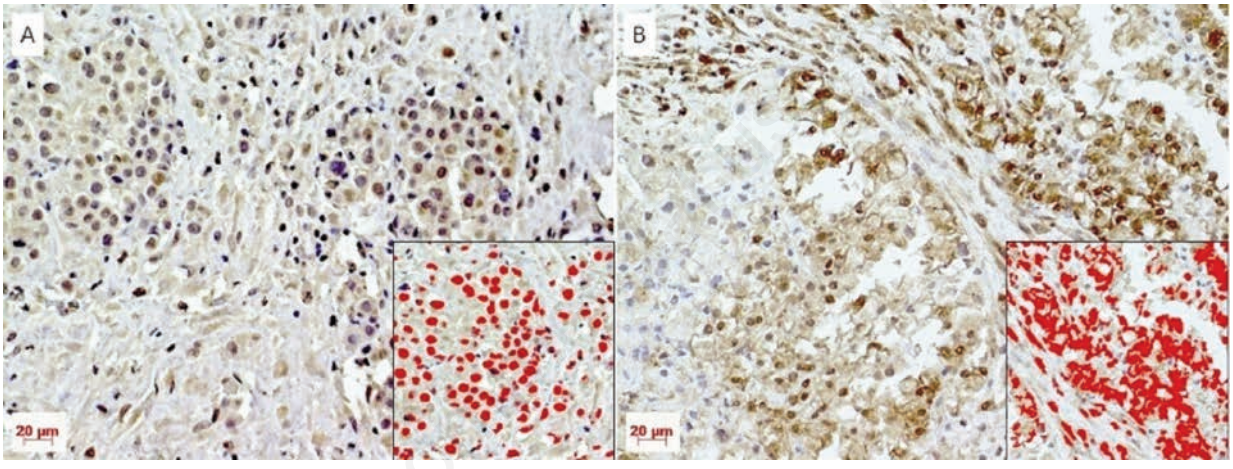


**Figure 2. A**) High immunohistochemical expression of PACAP in epithelioid MPM (immunoperoxidase staining); representative PACAP immunostaining software image analysis in epithelioid MPM (insert). **B**) Strong and diffuse immunostaining with PAC1R in epithelioid MPM (immunoperoxidase staining); representative PAC1R immunostaining software image analysis in epithelioid MPM (insert).

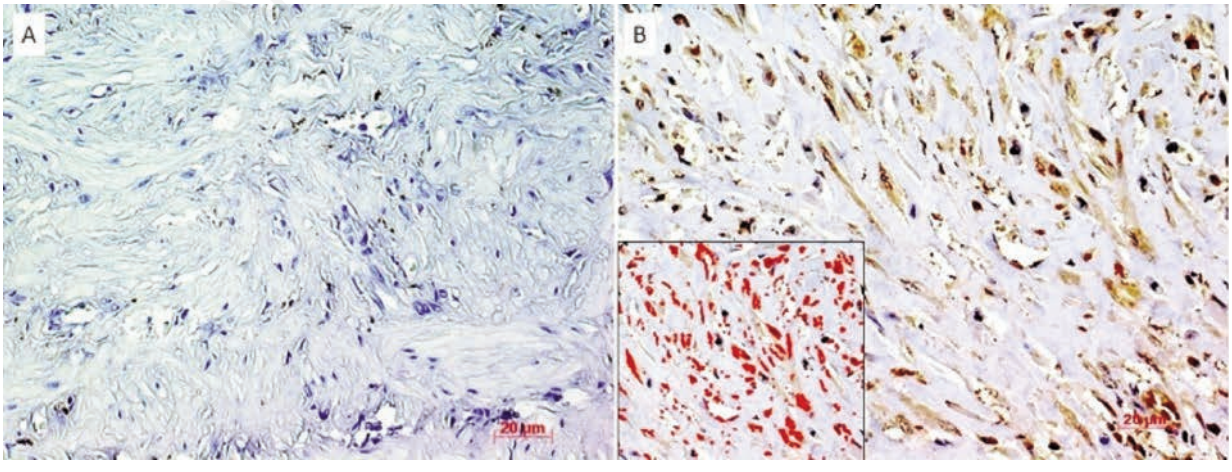


**Figure 3. A**) Lack of immunohistochemical expression of PACAP in sarcomatoid MPM (immunoperoxidase staining). **B**) Neoplastic cells of sarcomatoid MPM are diffusely and strongly stained with PAC1R (immunoperoxidase staining); representative PAC1R immunostaining software image analysis in sarcomatoid MPM (insert).

with $p<0.0001$. In Figure 5, the densitometric analysis of PACAP and PAC1R in MPMs tissue compared to the controls tissue were shown. In particular, Figure 5A showed the significantly increase of densitometric expression of PACAP in MPMs compared to the controls with $p=0.0049$; Figure 5B showed the significantly increase of morphometric expression of PAC1R in MPMs compared to the controls with $p=0.0005$. Comparing the morphometric expressions of PACAP and PAC1R of MPM cases, the results showed the greatest expression of PAC1R in all the subtypes of cancer (Figure 6). Two way ANOVA showed a statistically significant trend between rows ($p=0.0038$) and columns ($p<0.0001$). Comparing the densitometric expressions of PACAP and PAC1R of MPM cases, two way ANOVA showed a no statistically significant trend between rows ($p=0.0507$) and a statistically significant trend between columns ($p<0.0001$) (Figure 7). Both analyses showed negative PACAP expression in the case of the sarcomatoid histotype.

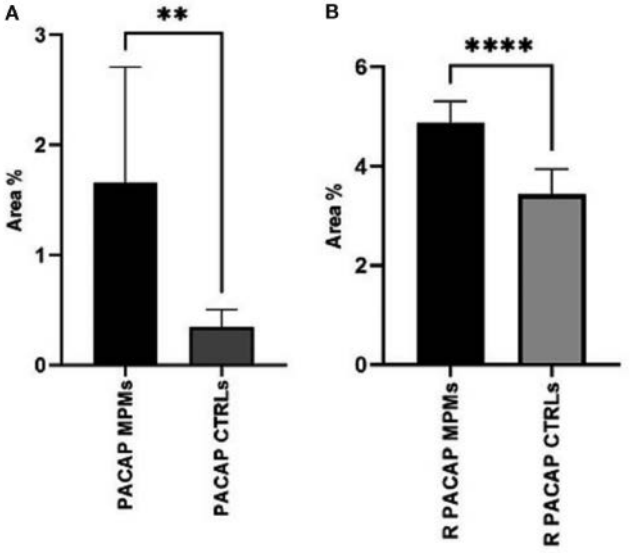


**Figure 4. A**) The significantly increase of morphometric expression of PACAP in MPMs compared to the controls with $p=0.0068$. **B**) The significantly increase of morphometric expression of PAC1R (R PACAP) in MPMs compared to the controls with $p<0.0001$.

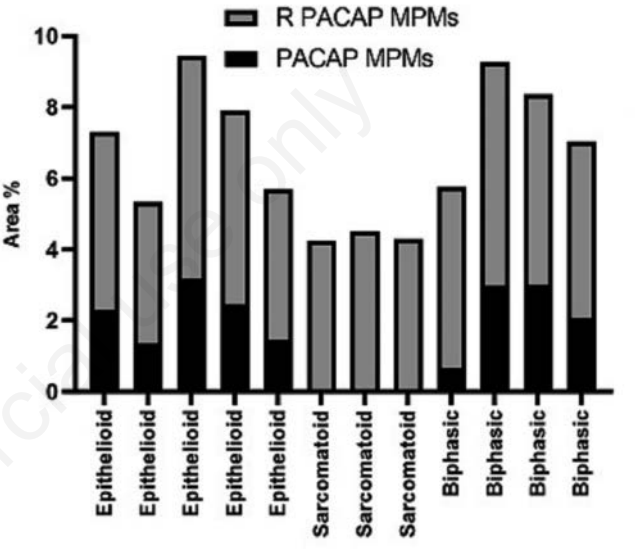


**Figure 6.** Morphometric expressions of PACAP and PAC1R (R PACAP) of MPM cases.

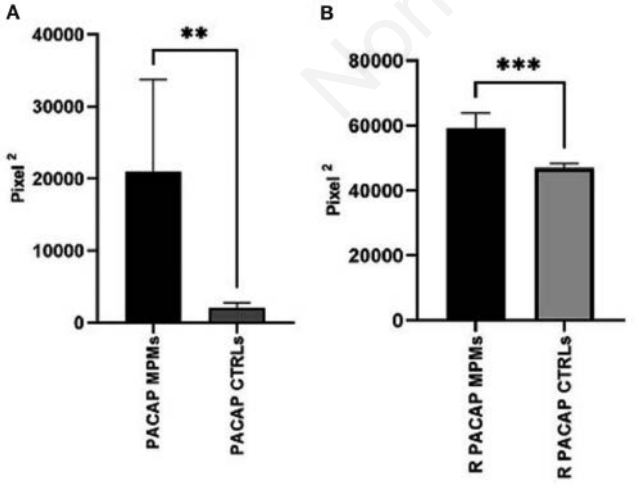


**Figure 5. A**) The significantly increase of densitometric expression of PACAP in MPMs compared to the controls with $p=0.0049$. **B**) The significantly increase of morphometric expression of PAC1R (R PACAP) in MPMs compared to the controls with $p=0.0005$.

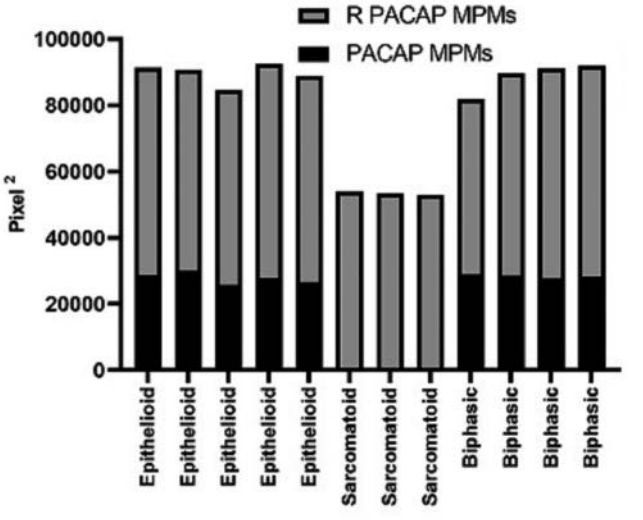


**Figure 7.** Densitometric expressions of PACAP and PAC1R (R PACAP) of MPM cases. PACAP) in MPMs compared to the controls with $p=0.0005$.

## Discussion

Pleural mesothelioma is an aggressive cancer that arises from the mesothelial cells lining the pleural cavity, characterized by poor life expectancy. The discovery of novel immunohistochemical markers in pleural mesothelioma represents a topic of great interest because it allows differential diagnosis with benign and metastatic tumors. The diagnosis of pleural mesothelioma based on histopathological characteristics can be limiting since it can often exhibit features similar to other malignancies. Therefore the identification of immunohistochemical markers is essential to ensure an accurate diagnosis.

PACAP is a neuropeptide isolated for the first time in 1989 from sheep hypothalamic extracts.[36] It exerts a central role in the development of the nervous system and different peripheral organs.[37] Different studies demonstrated its involvement in several neurodegenerative diseases, where the peptide has been shown to play a trophic and protective role.[38-43]

The role of PACAP in cancers is controversial, in fact, some tumors show high expression levels of the PACAP-ergic system, whereas others display downregulation of PACAP-PAC1R signaling. Moreover, the exogenous treatment with the peptide can promote or inhibit tumor cells growth.[44-46] The differential role played by PACAP is related to multiple aspects including tumor type and origin, differentiation stage, and tumor microenvironment.[47] In addition, it is necessary to point out that alternative splicing, verifying in *PAC1R* gene generates different variants (Null, Hip, Hop1, Hop2, Hiphop1, Hiphop2, short and very short isoforms). These isoforms can activate AC forming cAMP, or PLC pathway inducing the formation of protein kinase C (PKC), by leading differential mechanisms.[48]

In the present study we analysed MPM samples for PACAP and PAC1R immunostaining. Both PACAP and its preferring receptor PAC1, are strongly expressed in the epithelioid and biphasic subtypes. High expression levels of the PAC1R were also detected in the sarcomatoid sample, whereas strongly decreased peptide expression was found in the sarcomatoid subtype. Although further studies on larger series are needed to validate this latter finding, it seems very interesting since it potentially demonstrated that PACAP could be useful in subtype discrimination, confirming predilection for the epithelioid and biphasic subtypes.

Interestingly, there is a similar expression pattern between PACAP and epidermal growth factor receptor (EGFR) in the pleural mesothelioma subtypes. In fact, EGFR immunoreactivity was found with higher expression in the epithelial subtype as compared to the sarcomatoid one.[49-51] This could be in part due to the fact that PACAP via PAC1R activation induces the trans-activation of EGFR.[52,53]

The gold standard for pleural mesothelioma diagnosis and the discrimination of its subtypes is the immunohistological examination of conventionally stained tissue samples. In the present study, we identified PACAP as an innovative and useful marker for epithelioid and biphasic subtypes of pleural mesothelioma. In the future, we hope to confirm and expand on these data with increased numbers of cases.

## References

1. Robinson BW, Musk AW, Lake RA. Malignant mesothelioma. Lancet 2005;366:397-408.
2. Barone AD, Mastrantonio M, Romeo L. Environmental risk factors in pleural malignant mesothelioma. Medicina Lavoro 2012;103:29-42.
3. Ledda C, Loreto C, Lombardo C, Cardile V, Rapisarda V. Mesothelin methylation, soluble mesothelin related protein levels and inflammation profiling in workers chronically exposed to naturally occurring asbestos fibers. Transl Oncol 2024;40:101872.
4. Comba P, Gianfagna A, Paoletti L. Pleural mesothelioma cases in Biancavilla are related to a new fluoro-edenite fibrous amphibole. Arch Environ Health 2011;66:70-6.
5. Magnani C, Dalmasso P, Biggeri A. Increased risk of malignant mesothelioma of the pleura after residential or domestic exposure to asbestos: a case-control study in Casale Monferrato, Italy. Environ Health Perspect 1996;104:1208-13.
6. Fazz L, Minelli G, De Santis M. Pleural mesothelioma mortality and asbestos exposure mapping in Italy. Am J Ind Med 2015;58:1224-34.
7. Grosse Y, Loomis D, Guyton KZ, Lauby-Secretan B, El Ghissassi F, Bouvard V, et al. Carcinogenicity of fluoro-edenite, silicon carbide fibres and whiskers, and carbon nanotubes. Lancet Oncol 2014;15:1427-8.
8. Ballan G, Del Brocco A, Loizzo S, Fabbri A, Maroccia Z, Fiorentini C, Travaglione S. Mode of action of fibrous amphiboles: the case of Biancavilla (Sicily, Italy). Ann Ist Super Sanita 2014;50:133-8.
9. Filetti V, Vitale E, Broggi G, Hagnäs MP, Candido S, Spina A, et al. Update of in vitro, in vivo and ex vivo fluoro-edenite effects on malignant mesothelioma: A systematic review. Biomed Rep 2020;13:60.
10. Loreto C, Caltabiano R, Graziano ACE, Castorina S, Lombardo C, Filetti V et al. Defense and protection mechanisms in lung exposed to asbestiform fiber: the role of macrophage migration inhibitory factor and heme oxygenase-1. Eur J Histochem 2020;64:3073.
11. Filetti V, Loreto C, Falzone L, Lombardo C, Cannizzaro E, Castorina S, et al. Diagnostic and prognostic value of three microRNAs in environmental asbestiform fibers-associated malignant mesothelioma J Pers Med 2021;15;11:1205.
12. Sayan M, Mossman BT. The NLRP3 inflammasome in pathogenic particle and fibre-associated lung inflammation and diseases. Part Fibre Toxicol 2016;13:51.
13. Lombardo C, Broggi G, Vitale E, Ledda C, Loreto C, Matera S, et al. Expression of stathmin in asbestos-like fibers-induced mesothelioma: A preliminary report. Histol Histopathol 2023;38:1249-56.
14. Filetti V, Lombardo C, Loreto C, Dounias G, Bracci M, Matera S, et al. Small RNA-Seq transcriptome profiling of mesothelial and mesothelioma cell lines revealed microRNA dysregulation after exposure to asbestos-like fibers. Biomedicines 2023;11:538.
15. Ledda C, Lombardo C, Tendi EA, Hagnas M, Paravizzini G, Filetti V, et al. Pathway of inflammation due to asbestiform fiber "fluoro-edenite" exposure: an update. Curr Respir Med Rev 2020;16:73-5.
16. Rapisarda V, Caltabiano R, Musumeci G, Castrogiovanni P, Ferrante M, Ledda C, et al. Analysis of fibulin-3 after exposure to asbestos-like fibers Environ Res 2017;156:381-7.
17. Brody AR. How inhaled asbestos causes scarring and cancer. Curr Respir Med Rev 2018;14:204-17.
18. Broggi G, Filetti V, Magro G, Morante B, Garro R, Ledda C, et al. Immunohistochemical expression of cAMP in fluoroedenite induced malignant pleural mesothelioma: Preliminary results. Mol Med Rep 2023;28:132.
19. Rapisarda V, Broggi G, Caltabiano R, Lombardo C, Castorina S, Trovato A, et al. ATG7 immunohistochemical expression in malignant pleural mesothelioma. A preliminary report. Histol Histopathol 2021;36:1301-8.

20. Moody TW, Jensen RT. VIP and PACAP: recent insights into their functions/roles in physiology and disease from molecular and genetic studies. Curr Opin Endocrinol Diabetes Obes 2006;13:107-15.
21. Vaudry D, Falluel-Morel A, Bourgault S. Pituitary adenylate cyclase-activating polypeptide and its receptors: 20 years after the discovery. Pharmacol Rev 2009;61:283-357.
22. Arimura A, Shioda S. Pituitary adenylate cyclase activating polypeptide (PACAP) and its receptors: neuroendocrine and endocrine interaction. Front Neuroendocrinol 1995;16:53-88.
23. Arimura A. Perspectives on pituitary adenylate cyclase-activating polypeptide (PACAP) in the neuroendocrine, endocrine, and nervous systems. J Recept Signal Transduct Res 1998;18:533-65.
24. Waschek JA. VIP and PACAP: neuropeptide modulators of CNS inflammation, injury, and repair. Brit J Pharmacol 2002;137:1005-18.
25. D'Amico AG, Maugeri G, Magrì B, Lombardo C, Saccone S, Federico C, et al. PACAP-ADNP axis prevents outer retinal barrier breakdown and choroidal neovascularization by interfering with VEGF secreted from retinal pigmented epitelium cells. Peptides 2023;168:171065.
26. D'Amico AG, Maugeri G, Vanella L, Pittalà V, Reglodi D, D'Agata V. Multimodal role of PACAP in glioblastoma. Brain Sci. 2021;11:994.
27. Maugeri G, D'Amico AG, Saccone S, Federico C, Rasà DM, Caltabiano R, et al. Effect of PACAP on hypoxia-induced angiogenesis and epithelial-mesenchymal transition in glioblastoma. Biomedicines. 2021;9:965.
28. Maugeri G, D'Amico AG, Reitano R, Magro G, Cavallaro S, Salomone S, D'Agata V. PACAP and VIP inhibit the invasiveness of glioblastoma cells exposed to hypoxia through the regulation of HIFs and EGFR expression. Front Pharmacol 2016;7:139.
29. Maugeri G, D'Amico AG, Rasà DM, Saccone S, Federico C, Cavallaro S, D'Agata V. PACAP and VIP regulate hypoxia-inducible factors in neuroblastoma cells exposed to hypoxia. Neuropeptides 2018;69:84-91.
30. Noh KT, Kim HJ, Kim MS. PACAP inhibits tumor growth and interferes with clusterin in cervical carcinomas. Oncol Reports 2015;34:163-70.
31. Juhász T, Matta C, Katona É, Somogyi C, Takács R, Hajdú T, et al. Pituitary adenylate cyclase-activating polypeptide (PACAP) signalling enhances osteogenesis in UMR-106 cell line. J Mol Neurosci 2014;54:555-73.
32. Le SV, Yamaguchi DJ, McArdle CA, Tachiki K, Pisegna JR, Germano P. PAC1 and PACAP expression, signaling, and effect on the growth of HCT8, human colonic tumour cells. Regul Pept 2002;109:115-25.
33. Dacic S. Pleural mesothelioma classification-update and challenges. Mod Pathol 2022;35:51-6.
34. Broggi G, Angelico G, Filetti V, Ledda C, Lombardo C, Vitale E, et al. 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 2021;18:6249.
35. Loreto C, Lombardo C, Caltabiano R, Ledda C, Hagnas M, Filetti V, et al. In vivo immunohistochemical study on macroH2A.1 in lung and lymph-node tissues exposed to an asbestiform fiber. Curr Mol Med 2020;20:653-60.
36. Miyata A, Arimura A, Dahl RR, Minamino N, Uehara A, Jiang L, Culler MD, Coy DH. Isolation of a novel 38 residue-hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells. Biochem Biophys Res Commun.1989;164:567-74.
37. Sandor B, Fintor K, Reglodi D, Fulop DB, Helyes Z, Szanto I, et al. Structural and morphometric comparison of lower incisors in PACAP-deficient and wild-type mice. J Mol Neurosci 2016;59:300-8.
38. Solés-Tarrés I, Cabezas-Llobet N, Vaudry D, Xifró X. Protective effects of pituitary adenylate cyclase-activating polypeptide and vasoactive intestinal peptide against cognitive decline in neurodegenerative diseases. Front Cell Neurosci 2020;14:221.
39. Reglodi D, Kiss P, Lubics A, Tamas A. Review on the protective effects of PACAP in models of neurodegenerative diseases in vitro and in vivo. Curr Pharm Des 2011;17:962-72.
40. Waschek JA. VIP and PACAP: neuropeptide modulators of CNS inflammation, injury, and repair. Br J Pharmacol 2013;169:512-23.
41. D'Amico AG, Maugeri G, Saccone S, Federico C, Cavallaro S, Reglodi D, D'Agata V. PACAP modulates the autophagy process in an in vitro model of amyotrophic lateral sclerosis. Int J Mol Sci 2020;21:2943.
42. Maugeri G, D'Amico AG, Morello G, Reglodi D, Cavallaro S, D'Agata V. Differential vulnerability of oculomotor versus hypoglossal nucleus during ALS: involvement of PACAP. Front Neurosci 2020;14:805.
43. Maugeri G, D'Amico AG, Musumeci G, Reglodi D, D'Agata V. Effects of PACAP on Schwann cells: focus on nerve injury. Int J Mol Sci 2020;21:8233.
44. Reubi JC, Läderach U, Waser B, Gebbers JO, Robberecht P, Laissue JA. Vasoactive intestinal peptide/pituitary adenylate cyclase-activating peptide receptor subtypes in human tumors and their tissues of origin. Cancer Res 2000;60:3105-12.
45. Schulz S, Röcken C, Mawrin C, Weise W, Höllt V, Schulz S. Immunocytochemical identification of VPAC1, VPAC2, and PAC1 receptors in normal and neoplastic human tissues with subtype-specific antibodies. Clin Cancer Res 2004;10:8235-42.
46. Cochaud S, Meunier AC, Monvoisin A, Bensalma S, Muller JM, Chadéneau C. Neuropeptides of the VIP family inhibit glioblastoma cell invasion. J Neurooncol 2015;122:63-73.
47. Zibara K, Zeidan A, Mallah K, Kassem N, Awad A, Mazurier F, et al. Signaling pathways activated by PACAP in MCF-7 breast cancer cells. Cell Signal 2018;50:37-47.
48. Dickson L, Finlayson K. VPAC and PAC receptors: From ligands to function. Pharmacol Ther 2009;121:294-316.
49. Dazzi H, Hasleton PS, Thatcher N, Wilkes S, Swindell R, Chatterjee AK. Malignant pleural mesothelioma and epidermal growth factor receptor (EGF-R). Relationship of EGF-R with histology and survival using fixed paraffin embedded tissue and the F4, monoclonal antibody. Br J Cancer 1990;61:924-6.
50. Destro A, Ceresoli GL, Falleni M, Zucali PA, Morenghi E, Bianchi P, et al. EGFR overexpression in malignant pleural mesothelioma. An immunohistochemical and molecular study with clinico-pathological correlations. Lung Cancer 2006;51:207-15.
51. Chia PL, Parakh S, Russell P, Gan HK, Asadi K, Gebski V, et al. Expression of EGFR and conformational forms of EGFR in malignant pleural mesothelioma and its impact on survival. Lung Cancer 2021;153:35-41.
52. Maugeri G, D'Amico AG, Castrogiovanni P, Saccone S, Federico C, Reibaldi M, et al. PACAP through EGFR transactivation preserves human corneal endothelial integrity. J Cell Biochem 2019;120:10097-105.
53. Moody TW, Lee L, Iordanskaia T, Ramos-Alvarez I, Moreno P, Boudreau HE, et al. PAC1 regulates receptor tyrosine kinase transactivation in a reactive oxygen species-dependent manner. Peptides 2019;120:170017.

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