Immunohistochemical expression of cAMP in fluoroedenite-induced malignant pleural mesothelioma: Preliminary results

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Abstract. Despite advances in understanding of the biology of malignant pleural mesothelioma (MPM), the prognosis of this malignancy remains poor. Although asbestos still remains the main pathogenic agent of MPM, other asbestos-like fibers such as fluoro-edenite (FE) fibers, induce MPM. Notable incidence and mortality rates of MPM have been found in Biancavilla, Italy, where FE fibers have been extracted from building materials for >50 years. Cyclic adenosine monophosphate (cAMP) is a secondary messenger that plays a key role in several physiological and pathological mechanisms regulating protein kinase A (PKA) and the CREB pathway. Hyperactivation of the cAMP/PKA/CREB pathway is involved in many neoplastic processes, including tumor cell proliferation, invasion and metastatic spread. The present study investigated immunohistochemical expression of cAMP in patients with FE-induced MPM, which included six males and four females with an age range of 50-93 years. There was high immunoexpression of cAMP in 5 out of 10 tumors while the remaining 5 cases showed low immunoexpression. In addition, there was a correlation between cAMP overexpression and decreased survival times (mean overall survival times, 7.5 months in high expression group vs. 18 months in low expression group).

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Introduction

Malignant pleural mesothelioma (MPM) is a biologically aggressive malignancy arising from the pleural mesothelium and pathogenically linked to occupational or residential exposure to asbestos fibers (1,2). MPM has poor outcomes (median overall survival from 6 to 12 months) with late diagnosis and low treatment response (1,2).

Currently, the main clinicopathological features with prognostic relevance in patients with MPM include pathological stage, histological subtype, sex and age (3). Immunohistochemistry plays a leading role in the diagnosis of this neoplasm and Wilms' Tumor-1 (WT-1), calretinin, Cytokeratin 5/6 (CK5/6), podoplanin, mesothelin and osteopontin are immunomarkers currently available for MPM. However, in the last decade, clinicians have reported a potential prognostic and predictive role of proteins expressed in MPM, including caspase-3 (4), autophagy-related protein 7 (5) and serine and Arginine-rich splicing factor 1 (6); to the best of our knowledge, however, reliable prognostic and predictive biomarkers capable of improving MPM treatment and outcome have not yet been characterized.

In addition to asbestos, other pathogenic agents, including asbestos-like fibers such as erionite and fluoroedenite (FE) fibers, induce MPM (7-9). Several cases of MPM have been reported by previous epidemiological studies in Biancavilla, a small town near Mt. Etna (Sicily, Italy) (10-12); FE fibers were isolated in the lava rocks excavated from a local stone quarry that had been used to extract building materials for >50 years (10-12). A strong morphological and size overlap was demonstrated between the extracted building materials and tremolite amphibolic asbestos fibers and the International Agency for Research on Cancer declared FE fibers as environmental carcinogens (12).

Cyclic adenosine monophosphate (cAMP) was the first secondary messenger discovered and plays crucial roles in cell targeting and signaling, regulating physiological and pathological mechanisms (13,14). The transcription of

numerous genes may be regulated by cAMP via the classically kinase A (PKA) and cAMP-responsive element binding protein (CREB) pathway (13). PKA also serves as a phosphorylating agent of multiple kinases, including Raf, glycogen synthase kinase 3 (GSK3) and focal adhesion kinase (FAK) (13). The aberrant activation of the cAMP/PKA pathway has been shown to be involved in many human neoplasms including liver, brain, prostate, breast and lung cancer, regulating tumor cell proliferation, invasion and metastatic potential (13).

To the best of our knowledge, there is few data about the role of cAMP in MPM. Therefore, the present study investigated the immunohistochemical expression of cAMP and its correlation with the clinicopathological parameters in patients with MPM exposed to FE fibers.

Materials and methods

Sample collection. The present research complied with the Helsinki Declaration and was approved by the Catania 1 Ethics Committee, 'Policlinico-Vittorio Emanuele', Catania, Italy (approval no. 1/2014/PO; 28/05/2014).Written informed consent was obtained from all patients enrolled in the study. All patients were recruited from the University-Hospital Policlinico-Vittorio Emanuele.

Clinicopathological features from 49 surgically treated cases with a histological diagnosis of MPM diagnosed between January 1996 and December 2014 were collected from patients (28 males and 21 females; age range: 47-93 years) who were residents in Biancavilla and exhibited evidence of environmental exposure to FE. Adequate thoracoscopic biopsy tissue and follow-up data were available only for 10 of the 49 patients. Inclusion criteria for histological samples were as previously reported (5,6): i) Tumor tissue from the paraffin-embedded blocks had to be sufficient to cut slides for immunohistochemical analyses and ii) representative tumor tissue had to be present in paraffin-embedded blocks. Cases with extensive necrosis were excluded to preserve the immunoreactivity of the tumor tissue.

Immunohistochemistry. Surgical specimens were fixed in 10% formalin for 12-24 h, embedded in paraffin at 46-68˚C, cut to 4-5 μm and stained with 5% hematoxylin for 4 min and with 1-5% eosin for two min at room temperature. Each sample was incubated overnight at 4˚C with a rabbit monoclonal anti-cAMP protein kinase catalytic subunit antibody (clone EP2102Y; cat. no. ab76238; Abcam), diluted at 1:100 in PBS). The presence of brown chromogen within the tumor cytoplasm was assessed as positive cAMP staining; unaffected human testis tissue was used as a positive control, while negative control sections were obtained by omitting the primary antibody. cAMP immunoreactivity was evaluated within areas of vital tumor tissue, while necrotic areas were excluded from the analysis.

Immunohistochemical slides were semi-quantified as previously described (15,16). Immunoreactivity score (IRS) was obtained by multiplying the intensity of staining (IS; 0-3) and the percentage of positive cells (extent score, ES; 0-4). IRS ≤6 or >6 indicated low and high cAMP expression, respectively.

Statistical analysis. The mean and median values of cAMP expression, expressed as IRS, in FE-induced MPM were non-parametrically compared by χ^2 test. Hazard ratio (HR) was calculated using the Mantel-Haenszel test. Cancer-specific survival analysis and comparison of survival curves were performed using the Kaplan-Meier method and Mantel-Cox log-rank test, respectively. Spearman's correlation was performed to evaluate the correlation between clinicopathological and immunohistochemical data. $P<0.05$ was considered to indicate a statistically significant difference. Statistical analysis was performed using GraphPad Prism version 7 (GraphPad Software, Inc.).

Results

Clinicopathological features of the FE-induced MPM cases. The clinicopathological features were as previously described (6). Briefly, six males and four females affected by FE-induced MPM with an age range of 50-93 years (mean age, 68.4 years), were part of the study. According to the World Health Organization (WHO) criteria (17), histopathology showed an epithelioid morphology in six cases, a sarcomatoid morphology in one case and a biphasic morphology in the remaining three cases. A mild predominance of the sarcomatoid component (60 vs. 40% epithelioid) was observed in two biphasic MPMs, while an almost pure spindle cell morphology with only scattered glandular structures was seen in the remaining biphasic case. No signs of apoptosis were found. The primary clinicopathological and immunohistochemical features are summarized in Table I.

Immunohistochemical expression of cAMP and correlation with clinicopathological parameters. High immunohistochemical expression of cAMP was found in 5 tumors (50%; Fig. 1A and B), while the remaining 5 cases (50%) exhibited low immunoexpression (Fig. 1C and D).

Considering the median overall survival (OS) time between high (8 months) and low (18 months) cAMP expression, there was no significant association between cAMP expression and increased OS and HR was 0.226 (95% CI, 0.049-1.042; Fig. 2). No significant association between cAMP expression and other clinicopathological variables (age, sex and MPM pathological subtype) was observed (data not shown). Moreover, the better prognosis was observed in cases that exhibited a low immunoexpression of cAMP. By contrast, shorter OS was found in patients with FE-induced MPM with high cAMP expression. A correlation between cAMP overexpression and decreased survival time was found (mean OS, 7.5 for patients with high expression vs. 18.0 months for patients with low cAMP expression; Fig. 2).

Discussion

The present study investigated the immunohistochemical expression of cAMP in patients with MPM characterized by exposure to FE fibers. High cAMP immunoexpression in five MPM cases (50%), while the remaining 5 cases (50%) exhibited low immunoreactivity. In addition, a trend of shorter OS was found in patients with FE-induced MPM and high cAMP expression (mean OS time of 7.5 months for patients with high

Table I. Clinico-pathological and immunohistochemical features of the cases from our series.

Case	Age, years	Sex	Pathological subtype	Survival time, months	cAMP IS	cAMP ES	cAMP IRS
1	69	M	Epithelioid	2	2	4	8
2	50	M	Biphasic (20% epithelioid, 80% sarcomatoid)	16	2	2	4
3	69	F	Sarcomatoid	5	3	3	9
4	74	F	Epithelioid	13	2	2	4
5	85	M	Epithelioid	23	2	4	8
6	93	F	Biphasic (40% epithelioid, 60% sarcomatoid)	8	3	4	12
7	58	F	Epithelioid	18	2	2	4
8	55	M	Epithelioid	37	1	2	2
9	75	M	Biphasic (40% epithelioid, 60% sarcomatoid)	60	2	2	4
10	56	M	Epithelioid	12	2	4	8

M, male; F, female; IS, intensity of staining; ES, extent score; IRS, immunoreactivity score.

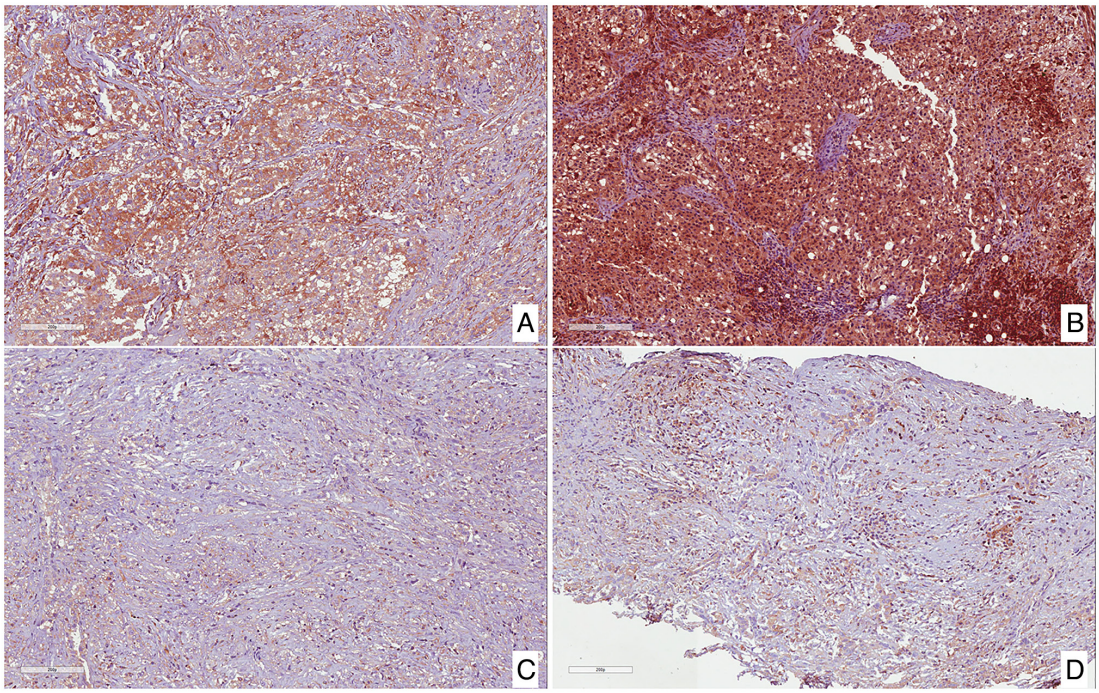

Figure 1. Immunohistochemical expression of cAMP. (A) High immunohistochemical expression of cAMP in a case of epithelioid MPM. (B) High immunohistochemical expression of cAMP in a second case of epithelioid MPM. (C) Low immunohistochemical expression of cAMP in a case of biphasic MPM. (D) Low immunohistochemical expression of cAMP in a second case of biphasic MPM. Magnification, x200x. MPM, malignant pleural mesothelioma.

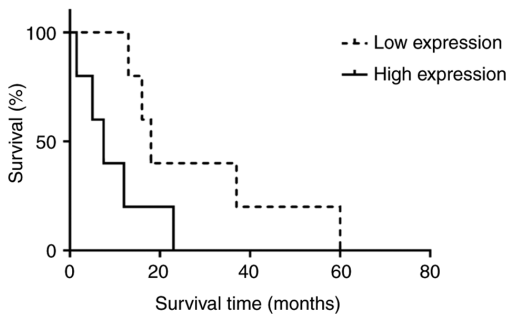

Figure 2. Survival curves in patients with high and low cAMP expression. Kaplan-Meier survival curve based on cAMP expression in patients with fluoroedenite-induced malignant pleural mesothelioma (P=0.0565).

expression vs. mean OS of 18 months for patients with low cAMP expression).

cAMP acts as an intracellular secondary messenger, by regulating several processes such as cellular metabolism, ion channel activation, gene transcription, cell proliferation, differentiation, cell death and apoptosis (13,14). cAMP may play both a tumor-suppressive and tumor-promoting role by interacting with CREB and PKA, which induces the phosphorylation of multiple kinases, including Raf, GSK3 and FAK (13). In hepatocellular carcinoma, the role of cAMP in tumorigenesis is controversial because some authors suggested that increasing cAMP levels inhibits cell proliferation (18,19), while some clinicians demonstrated

that PKA promotes tumor invasion and metastasis via phosphorylation of Cdc42-interacting protein 4 (CIP4) (20). In brain tumors, cAMP has been found to inhibit glioblastoma and medulloblastoma cell proliferation by increasing p21/p27 and PKA/exchange protein activated by cAMP (Epac1)/Rap1 signaling (21,22) and decreasing Hedgehog pathway signaling (23,24), respectively. It has also been suggested that PKA overexpression may negatively influence the androgen receptor signaling status of prostate cancer, leading to the development of androgen resistance and tumor growth (25,26). Similarly, the cAMP/PKA axis has been found to stimulate tamoxifen resistance and tumor progression in estrogen receptor-positive breast cancer (27) and trastuzumab resistance in HER-2 positive breast cancer (28).

Although the involvement of cAMP in tumorigenesis and tumor growth has been reported in various human neoplasms (13), little is known about its role in MPM.

In the past few decades, some clinicians have found that asbestos fibers rapidly induced CREB1 phosphorylation and upregulation of CREB target genes, including c-Fos, Early Growth Response-1 (EGR-1), Mitogen-activated protein kinase phosphatase 1 (MKP1), BCL2 and MMP13, on human mesothelial cell lines (29). Levels of phosphorylated CREB1 and mRNA of BCL2, c-FOS, MMP9 and MMP13 were found to be increased also in malignant mesothelioma cell lines (29). Therefore it is hypothesized that cAMP is upregulated in human mesothelium exposed to asbestos via the CREB pathway and may represent a novel potential therapeutic target for MPM treatment (29).

Aromatase may be involved in MPM tumorigenesis via modulation of cAMP (30,31), Nuvoli *et al* (32) found that exemestane, an inhibitor of aromatase, has beneficial effects in a preclinical model of MPM; specifically, exemestane negatively modulates expression of CD44 by downregulating cAMP and CREB and inhibits MPM cell proliferation (32).

Given the controversial role of cAMP in tumorigenesis, it is likely that its impact is associated with specific tissue type and other factors on which patient prognosis typically depends, including tumor stage and microenvironment (tumor-associated inflammation and fibroblast levels and changes in tumor extracellular matrix composition) (33).

The present results are in line with the aforementioned data that attribute a tumor-promoting role to cAMP in MPM; the present study found a trend of shorter OS in patients with FE-induced MPM with high immunoexpression of cAMP. However, the present study is limited by the relatively small cohort of patients and the lack of statistical significance between cAMP expression and OS. Larger sample size is required to determine if there is a statistically significant association between cAMP and shorter OS. Although very few targeted therapies have been discovered and introduced in MPM treatment guidelines, patients with coactivation of AXL and Mesenchymal-epithelial transition factor (MET) tyrosine kinase receptors benefit from a targeted treatment with tyrosine kinase inhibitors (34). Future studies should assess the correlation between cAMP expression and co-activation of AXL and MET tyrosine kinase receptors and validate the present findings in animal models and/or MPM cell lines by *in vitro* experiments.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

GB and VF performed immunohistochemical experiments and statistical analysis. GB, GM, BM, RG and RC performed the histological examination. GB wrote the manuscript. CLom, VR, CLor and CLed analyzed and interpreted data. CLom, VR, CLor and CLed confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study complied with the Helsinki Declaration and was approved by the Catania 1 Ethics Committee, 'Policlinico-Vittorio Emanuele', Catania, Italy (approval no. 1/2014/PO; 28/05/2014). Written informed consent was obtained from the patients enrolled in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Perera ND and Mansfield AS: The evolving therapeutic landscape for malignant pleural mesothelioma. Curr Oncol Rep 24: 1413-1423, 2022.
2. Sinn K, Mosleh B and Hoda MA: Malignant pleural mesothelioma: Recent developments. Curr Opin Oncol 33: 80-86, 2021.
3. Delgermaa V, Takahashi K, Park EK, Le GV, Hara T and Sorahan T: Global mesothelioma deaths reported to the world health organization between 1994 and 2008. Bull World Health Organ 89: 716-724, 2011.
4. Rapisarda V, Ledda C, Migliore M, Salemi R, Musumeci A, Bracci M, Marconi A, Loreto C and Libra M: FBLN-3 as a biomarker of pleural plaques in workers occupationally exposed to carcinogenic fibers: A pilot study. Future Oncol 11: 35-37, 2015.
5. Rapisarda V, Broggi G, Caltabiano R, Lombardo C, Castorina S, Trovato A, Ledda C, Filetti V and Loreto C: ATG7 immunohistochemical expression in malignant pleural mesothelioma. A preliminary report. Histol Histopathol 36: 1301-1308, 2021.
6. Broggi G, Angelico G, Filetti V, Ledda C, Lombardo C, Vitale E, Rapisarda V, Loreto C and Caltabiano R: 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, 2021.

7. Filetti V, Vitale E, Broggi G, Hagnäs MP, Candido S, Spina A and Lombardo C: Update of in vitro, in vivo and ex vivo fluoro-edenite effects on malignant mesothelioma: A systematic review (Review). Biomed Rep 13: 60, 2020.
8. Ledda C, Loreto C, Pomara C, Rapisarda G, Fiore M, Ferrante M, Bracci M, Santarelli L, Fenga C and Rapisarda V: Sheep lymph-nodes as a biological indicator of environmental exposure to fluoro-edenite. Environ Res 147: 97-101, 2016.
9. Filetti V, Loreto C, Falzone L, Lombardo C, Cannizzaro E, Castorina S, Ledda C and Rapisarda V: Diagnostic and prognostic value of three microRNAs in environmental asbestiform fibers-associated malignant mesothelioma. J Pers Med 11: 1205, 2021.
10. Paoletti L, Batisti D, Bruno C, Di Paola M, Gianfagna A, Mastrantonio M, Nesti M and Comba P: Unusually high incidence of malignant pleural mesothelioma in a town of eastern Sicily: An epidemiological and environmental study. Arch Environ Health 55: 392-398, 2000.
11. Comba P, Gianfagna A and Paoletti L: Pleural mesothelioma cases in Biancavilla are related to a new fluoro-edenite fibrous amphibole. Arch Environ Health 58: 229-232, 2003.
12. Grosse Y, Loomis D, Guyton KZ, Lauby-Secretan B, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Scoccianti C, Mattock H, *et al*: Carcinogenicity of fluoro-edenite, silicon carbide fibres and whiskers, and carbon nanotubes. Lancet Oncol 15: 1427-1428, 2014.
13. Zhang H, Kong Q, Wang J, Jiang Y and Hua H: Complex roles of cAMP-PKA-CREB signaling in cancer. Exp Hematol Oncol 9: 32, 2020.
14. Jackson EK: The 2',3'-cAMP-adenosine pathway. Am J Physiol Renal Physiol 301: F1160-F1167, 2011.
15. Broggi G, Lo Giudice A, Di Mauro M, Pricoco E, Piombino E, Ferro M, Caltabiano R, Morgia G and Russo GI: Insulin signaling, androgen receptor and PSMA immunohistochemical analysis by semi-automated tissue microarray in prostate cancer with diabetes (DIAMOND study). Transl Res 238: 25-35, 2021.
16. Broggi G, Lo Giudice A, Di Mauro M, Asmundo MG, Pricoco E, Piombino E, Caltabiano R, Morgia G and Russo GI: SRSF-1 and microvessel density immunohistochemical analysis by semi-automated tissue microarray in prostate cancer patients with diabetes (DIAMOND study). Prostate 81: 882-892, 2021.
17. Galateau-Salle F, Churg A, Roggli V, Travis WD and World Health Organization Committee For Tumors Of The Pleura: The 2015 world health organization classification of tumors of the pleura: Advances since the 2004 classification. J Thorac Oncol 11: 142-154, 2016.
18. Massimi M, Ragusa F, Cardarelli S and Giorgi M: Targeting cyclic AMP signalling in hepatocellular carcinoma. Cells 8: 1511, 2019.
19. Massimi M, Cardarelli S, Galli F, Giardi MF, Ragusa F, Panera N, Cinque B, Cifone MG, Biagioni S and Giorgi M: Increase of intracellular cyclic AMP by PDE4 inhibitors affects HepG2 cell cycle progression and survival. J Cell Biochem 118: 1401-1411, 2017.
20. Tonucci FM, Almada E, Borini-Etichetti C, Pariani A, Hidalgo F, Rico MJ, Girardini J, Favre C, Goldenring JR, Menacho-Marquez M and Larocca MC: Identification of a CIP4 PKA phosphorylation site involved in the regulation of cancer cell invasiveness and metastasis. Cancer Lett 461: 65-77, 2019.
21. Chen TC, Hinton DR, Zidovetzki R and Hofman FM: Up-regulation of the cAMP/PKA pathway inhibits proliferation, induces differentiation, and leads to apoptosis in malignant gliomas. Lab Invest 78: 165-174, 1998.
22. Moon EY, Lee GH, Lee MS, Kim HM and Lee JW: Phosphodiesterase inhibitors control A172 human glioblastoma cell death through cAMP-mediated activation of protein kinase A and Epac1/Rap1 pathways. Life Sci 90: 373-380, 2012.
23. Cohen JR, Resnick DZ, Niewiadomski P, Dong H, Liau LM and Waschek JA: Pituitary adenylyl cyclase activating polypeptide inhibits gli1 gene expression and proliferation in primary medulloblastoma derived tumor sphere cultures. BMC Cancer 10: 676, 2010.
24. Ge X, Milenkovic L, Suyama K, Hartl T, Purzner T, Winans A, Meyer T and Scott MP: Phosphodiesterase 4D acts downstream of Neuropilin to control Hedgehog signal transduction and the growth of medulloblastoma. Elife 4: e07068, 2015.
25. Neary CL, Nesterova M, Cho YS, Cheadle C, Becker KG and Cho-Chung YS: Protein kinase A isozyme switching: Eliciting differential cAMP signaling and tumor reversion. Oncogene 23: 8847-8856, 2004.
26. Pollack A, Bae K, Khor LY, Al-Saleem T, Hammond ME, Venkatesan V, Byhardt RW, Asbell SO, Shipley WU and Sandler HM: The importance of protein kinase A in prostate cancer: Relationship to patient outcome in radiation therapy oncology group trial 92-02. Clin Cancer Res 15: 5478-5484, 2009.
27. Kok M, Zwart W, Holm C, Fles R, Hauptmann M, Veer LJ, Wessels LF, Neefjes J, Stål O, Linn SC, *et al*: PKA-induced phosphorylation of ERα at serine 305 and high PAK1 levels is associated with sensitivity to tamoxifen in ER-positive breast cancer. Breast Cancer Res Treat 125: 1-12, 2011.
28. Moody SE, Schinzel AC, Singh S, Izzo F, Strickland MR, Luo L, Thomas SR, Boehm JS, Kim SY, Wang ZC and Hahn WC: PRKACA mediates resistance to HER2-targeted therapy in breast cancer cells and restores anti-apoptotic signaling. Oncogene 34: 2061-2071, 2015.
29. Shukla A, Bosenberg MW, MacPherson MB, Butnor KJ, Heintz NH, Pass HI, Carbone M, Testa JR and Mossman BT: Activated cAMP response element binding protein is overexpressed in human mesotheliomas and inhibits apoptosis. Am J Pathol 175: 2197-2206, 2009.
30. Stoppoloni D, Salvatori L, Biroccio A, D'Angelo C, Muti P, Verdina A, Sacchi A, Vincenzi B, Baldi A and Galati R: Aromatase inhibitor exemestane has antiproliferative effects on human mesothelioma cells. J Thorac Oncol 6: 583-591, 2011.
31. Nuvoli B and Galati R: Cyclooxygenase-2, epidermal growth factor receptor, and aromatase signaling in inflammation and mesothelioma. Mol Cancer Ther 12: 844-852, 2013.
32. Nuvoli B, Germoni S, Morosetti C, Santoro R, Cortese G, Masi S, Cordone I and Galati R: Exemestane blocks mesothelioma growth through downregulation of cAMP, pCREB and CD44 implicating new treatment option in patients affected by this disease. Mol Cancer 13: 69, 2014.
33. Li Q, Wang W, Yamada T, Matsumoto K, Sakai K, Bando Y, Uehara H, Nishioka Y, Sone S, Iwakiri S, *et al*: Pleural mesothelioma instigates tumor-associated fibroblasts to promote progression via a malignant cytokine network. Am J Pathol 179: 1483-1493, 2011.
34. Marino FZ, Corte CM, Ciaramella V, Erra S, Ronchi A, Fiorelli A, Vicidomini G, Santini M, Scognamiglio G, Morgillo F, *et al*: AXL and MET tyrosine kinase receptors co-expression as a potential therapeutic target in malignant pleural mesothelioma. J Pers Med 12: 1993, 2022.