

FLUORO EDENITE-ASSOCIATED PATHOGENESIS IN PLEURAL MALIGNANT MESOTHELIOMA

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ABSTRACT

Aim: Malignant Pleural Mesothelioma (MPM) is a rare, but very aggressive tumour arising from pleural mesothelial cells. MPM etiology is strongly associated with exposure to asbestiform mineral fibres, which represent the principal carcinogenic factor. Inhalation of these fibres is interconnected with two pathogenic processes: chronic inflammation and carcinogenesis. Recently, an evident cluster of MPM cases was found in Biancavilla, an eastern Sicily village, where the onset of the lung tumour was associated with the exposure to the newly discovered asbestiform fibre, called Fluoro-Edenite (FE). This minireview focuses on the several studies, both *in vitro* and *in vivo*, that have been carried out until today, to understand the pathogenesis of MPM associated with the exposure to FE.

Materials and methods: The literature search was conducted on PubMed, Scopus and Google Scholar using appropriate keywords in relation to the pathogenesis related to Fluoro-edenite exposure.

Discussion: Previous studies reported that the biological reactivity of FE fibres resembles that of asbestos ones, and the exposure to these fibres could be associated with the onset of MPM. Until today, it has been discovered that the exposure to FE fibres induces the production of reactive oxygen and nitrogen species, which leads to cells death, chronic inflammation and aneuploidy. The carcinogenesis is therefore based on a close relationship between inflammatory process, DNA damage and apoptosis, which leads to the classic honeycombing of alveolar cells and fibrosis, but the mechanisms remain to be further elucidated.

Conclusion: Understanding of how pleural mesothelial cells respond to the FE fibres might be helpful to design preventive approaches to protect population from consequences of exposure to this airborne mineral fibre and may have important implications for therapeutic strategies.

Key words: Fluoro-edenite, Pleural Malignant Mesothelioma, Mineral fibres, Lung epithelial cells disease.

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Introduction

Pleura is a coelomic-derived serous membrane composed of mesothelial cells and loose connective tissue. Malignant Pleural Mesothelioma (MPM) is a rare, but rapidly fatal and aggressive tumour arising from pleural mesothelium with limited knowledge of its natural history. The earliest mention of the MPM was made in 1767 by Joseph Lieutaud, the founder of anatomic pathology in France, who found two cases of "pleural tumours" in a study of 3000 autopsies.

In 1819, René-Théophile-Hyacinthe Laennec, the French physician, based on his understanding of the nature of pleural cells, suggested the origin from the pleura, a thin membrane of lubricating cells, that lines the lungs and chest wall⁽¹⁾. MPM is divided into three major histological sub-types: epithelioid, sarcomatoid, and mixed (biphasic). Epithelioid subtype is the most common among patients with MPM (<50%), and it is also associated with a relative better prognosis⁽²⁾. Epithelioid mesothelioma can show a variable mixture of growth patterns, including tubule-papillary, adeno-

matoid (microglandular) (Fig. 1 A-C), and sheet-like patterns. Sarcomatoid mesothelioma is composed of spindle cells haphazardly arranged or exhibiting a fascicular growth pattern. Biphasic mesothelioma contains both epithelioid and sarcomatoid patterns, but this definition is better restricted to those cases in which one of the two components represents, at least, 10% of the entire tumour.

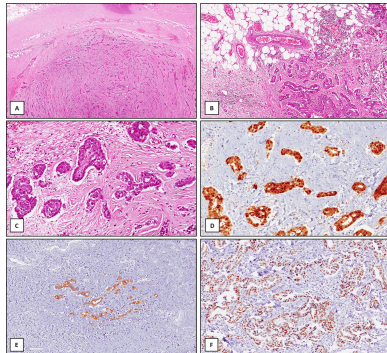


Fig. 1: Histological and immunohistochemical features of human pleural mesothelioma.

A). Epithelioid-type mesothelioma infiltrating parietal pleura. Magnification X50. **B).** Neoplastic glandular structures infiltrating fatty component of parietal pleura. Magnification X100. **C).** Higher magnification showing adenocarcinoma-like features of mesothelioma. Magnification X150. **D).** Neoplastic cells of mesothelioma showing both nuclear and cytoplasmic staining for calretinin. Magnification X100. **E).** Neoplastic cells exhibiting cytoplasmic staining for cytokeratins 5/6. Magnification X50. **F).** Neoplastic cells showing diffuse nuclear staining for WT1 (Wilm'tumour) protein. Magnification X50.

Although morphological diagnosis is usually straightforward, especially in biphasic tumours, differential diagnostic problems may arise when dealing with a “pure” epithelioid-type mesothelioma showing glandular growth pattern. This is mainly due to the fact that this latter variant poses serious diagnostic problems with pleural metastatic carcinoma, usually arising from lung. In this regard, immunohistochemical analysis, using several markers, is helpful in confirming diagnosis. Among these markers, the most reliable ones are calretinin, cytokeratins 5/6 and Wilm'tumour gene-1 (WT1).

This latter marker is highly sensitive and specific marker when evaluating a malignant tumour localized to pleura. WT1 nuclear expression has been reported in developmental human mesothelial cells⁽³⁾ and it is also retained in their malignant counterpart in mesothelioma. As this marker may be expressed also in the cytoplasm of several developing and neoplastic (benign and malignant) tis-

sues^(4,5), it should be emphasized that only WT1 nuclear expression is diagnostic of mesothelioma in the appropriate clinical and pathological context. (Fig. 1 D-F).

MPM etiology is strongly associated with exposure to asbestiform mineral fibres, which represent the principal carcinogenic factor. Inhalation of asbestiform mineral fibres, such as Fluoro-Edenite (FE), can induce two different, but interconnected, pathogenic processes involving lung tissue: chronic inflammation and carcinogenesis (Fig. 2).

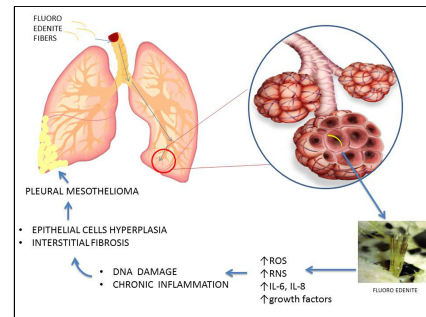


Fig. 2: Fluoro edenite-associated pathogenesis.

Inhaled Fluoro edenite fibres penetrate in the lung, reaching the peripheral air spaces where they are incorporated by alveoli, inducing the release of reactive oxygen and nitrite species (ROS and RNS) that results in DNA damage, and of cytokines (IL-6 and IL-8) and growth factors, which results in chronic inflammation process and epithelial cell proliferation. The last phases of the healing process are accompanied by fibrosis, triggering tumor development.

Both tissue alterations are mainly due to the fibres ability to stimulate host cell proliferation, as well as to induce the release of cytokines, growth factors and reactive oxygen and nitrite species (ROS and RNS), that results in DNA damage⁽⁶⁾. The microscopic data indicate that the early pathological event is caused by a direct mechanism, primarily acting at the alveolar level and, subsequently, at the interstitial one, and eventually inducing, in the last phases, a classic honeycombing. Inhaled asbestiform mineral fibres penetrate in the lung, reaching the peripheral air spaces where they are incorporated by alveolar macrophages, triggering an inflammatory response. The early phase of this response consists of the accumulation of macrophages in the alveolar ducts and peribronchiolar regions of the terminal respiratory bronchioles, followed by their accumulation, along with fibroblasts, in the pulmonary interstitium which results thickened. Migrating mineral fibres and oxidants released by activated macrophages, damage also adjacent cells, including type I alveolar epithelial cells, disrupting epithelial integrity and allowing

access of growth factors and cytokines to the interstitium. As part of the healing process, type II epithelial cell hyperplasia develops, accompanied by interstitial fibrosis with deposition of extracellular matrix proteins⁽⁷⁾.

Asbestos miners, insulation, shipyard, construction and heating trades workers are at greatest risk. Other factors which predispose to development of MPM are radiation therapy, tuberculosis, and chronic empyema. On imaging, diffuse nodular pleural thickening, pleural plaques, and pleural effusion are usually seen. The latent period for pleural plaque formation is usually 20 years and the presence of pleural plaques is a strong indicator of asbestiform fibres exposure. Pleurae, along the intercostal spaces, costophrenic angles, and lung apices are less frequently involved. Large pleural effusion, without mediastinal shift, may also be seen. Initial imaging modalities are chest radiography and Computed Tomography (CT). Further characterization may be required, using ultrasound (US) imaging, Magnetic resonance Imaging (MRI) and Positron Emission Tomography-CT (PET-CT). Biopsy remains the gold standard diagnostic technique⁽⁸⁾. It is possible to detect space-occupying lesions of the pleura, pleural effusion, focal or diffuse pleural thickening and subpleural lesions of the lung (Fig. 3), even in emergency settings.

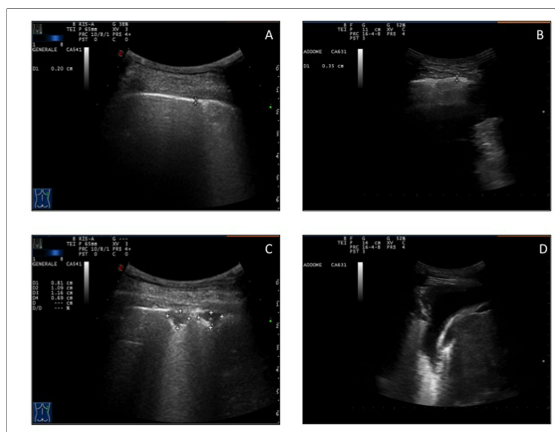


Fig. 3: Ultrasound thoracic imaging. **A).** Normal pleural line. **B).** Pleural thickening. **C).** Subpleural lesions. **D).** Pleural effusion.

Furthermore, transthoracic US imaging is useful as a guidance system for thoracentesis and peripheral lesion biopsy, where it minimises the occurrence of pneumothorax and haemorrhage⁽⁹⁾.

Epidemiological investigations (carried out from 1988 to 1992) on the mortality due to MPM in Italy⁽¹⁰⁾ showed a cluster of cases of this aggressive

tumour in Biancavilla, an eastern Sicily village in the province of Catania, located in the Etnean Volcanic Complex. In the absence of occupational risk factors connected with asbestos inhalation, the possible cause of the increased rate of mortality due to MPM, was proposed to be the stone quarries located in “Monte Calvario”, in the southeast of Biancavilla. All the population, in fact, was exposed to stone quarries, being this material usually used in the local building industry.

Among the materials present in the stone quarries, a new amphibole, named fluoro-edenite ($\text{NaCa}_2\text{Mg}_5\text{Si}_7\text{AlO}_{22}\text{F}_2$), was discovered⁽¹¹⁾. This asbestiform mineral fibre was recognized as a new mineral species in 2001, by the Commission on New Minerals and Mineral Names (IMA: code 2000-049)⁽¹²⁾. FE is now widely accepted as the main cause of MPM in Biancavilla, but further investigations for a more advanced knowledge of the step-by-step process, leading from early increased mesothelial cell proliferation to invasive mesothelioma, are still required. During the last years, several studies, both in vitro and in vivo, were carried out to understand the pathogenesis of MPM associated with the exposure to FE (Table 1).

IN VITRO STUDIES	FLUORO EDENITE EXPOSURE
Rapisarda et al. 2003	↑ROS
Cardile et al. 2004	↑ROS, ↑LDH, ↑NO
Travaglione et al. 2006	↑IL-6, ↑IL-8
Cardile et al. 2007	↑ROS, ↑RNS, ↑Hsp70
Pugnaloni et al. 2007	↑VEGF, ↑β-catenin, ↑COX-2, ↑PGE-2
Loreto et al. 2009	↑PLC
Musumeci et al. 2011	↑pRb
IN VIVO STUDIES	FLUORO EDENITE EXPOSURE
Martinez et al. 2006	↑TRAIL, ↑DR5, ↑MMP-13
Loreto et al. 2008	↓bcl-2, ↑bax
Musumeci et al. 2011	↑pRb
Rapisarda et al. (in press)	Pleural Plaques formation

Table 1: The table represents the summary of all the molecules studied until now, both in vitro and in vivo, in relation to exposure to fluoro edenite fibres and their respective consequences. The literature search on the topic was conducted on PubMed, Scopus and Google Scholar, using appropriate keywords in relation to fluoro-edenite and malignant pleural mesothelioma.

Materials and methods

In this narrative review, we analysed articles from the most recent literature, providing a bal-

anced and comprehensive overview of the most important discoveries in relation to the pathogenesis of MPM related to FE fibres exposure. Subsequently the selected articles were divided in “in vitro” and “in vivo” studies, to make the review more understandable and giving to the interested researchers a detailed and schematic overview of all the studies done about FE fibres. The literature search started in November 2013 on PubMed, Scopus and Google Scholar using the keywords ‘Fluoro-Edenite Fibres’, ‘Pleural Malignant Mesothelioma’ and ‘Mineral Fibres’ and out of approximately 80 papers only 37 have been chosen and considered appropriate for the aim of the review. The bibliographic research has been divided into 3 different steps and has followed an inductive reasoning (Table 2). In the first step, the research was focused on papers regarding FE fibres, both in vitro and in vivo, and on the analysis of the obtained data.

BIBLIOGRAPHIC RESEARCH	N° ACCEPTED REFERENCES (ref. List)	N° DISCHARGED REFERENCES
FE fibres in vitro studies	12 (from 13 to 24)	none
FE fibres in vivo studies	7 (from 25 to 31)	none
MPM	15 (from 1 to 9 and from 32 to 37)	35
Epidemiological studies	3 (from 10 to 12)	8

Table 2: The table represents the criteria used in the bibliographic research. The latter has been divided in 3 steps (FE fibres - in vitro and in vivo studies, MPM and Epidemiological studies) and followed an inductive reasoning. The table reports the number of references that have been accepted to be used in the review and the number of references that have been discharged out of the 80 publications initially chosen. Moreover, we report the specific references from the reference list, for every step of the bibliographic research.

In the second step, we chose the most updated and complete articles concerning MPM and its pathogenesis related to mineral fibres exposure. In this step we hypothesized a possible correlation between the exposure to FE fibres and the onset of MPM. In the latest step, we chose the articles concerning some recent epidemiologic studies. Other papers, although selected with the appropriate keywords, have been discharged, as considered outside the scope of the research, which wanted to be closely focused on FE fibres and their implication in the pathogenesis of MPM.

Retrospective analysis of the in vitro studies

The first in vitro study, carried out by Rapisarda et al.⁽¹³⁾ in 2003, concerned the ability of FE fibres to release hydroxyl radicals in vitro. The authors observed that the biological reactivity of these fibres resembled asbestos ones. They hypothesized also that the production of ROS by FE fibres, could mediate inflammatory fibrosis of the lung and DNA damage that may ultimately result in lung carcinoma and mesothelioma, as in the asbestos-mediated pathogenicity⁽¹⁴⁾. These data were then confirmed by Cardile et al.⁽¹⁵⁾, which reported that FE could affect some biochemical parameters in human lung alveolar epithelial cancer cell line (A549), human lung fibroblasts and monocyte-macrophage cell line (J774) exposed to the FE fibres, in concentration and/or treatment time dependent manner. In fact, at increasing FE concentrations, and/or treatment times, the increase in ROS production and lactate dehydrogenase (LDH) release was observed, underlining, again, the harmfulness of the fibre. Further studies investigated the involvement of nitric oxide (NO) in the cytotoxic and genotoxic effects caused by FE in J774 cell line, which resulted to be increased after high doses and longtime treatments, particularly in cultures treated with Lipopolysaccharides (LPS), demonstrating that inflammatory disorders increase the risk for lung cancer induced by FE and by the involvement of NO⁽¹⁶⁾.

Moreover, studies related to cellular transformation as a result of fibrous FE exposure, was carried out by Travaglione et al.⁽¹⁷⁾, who observed that fibrous FE induces dramatic changes in the lung epithelial cell morphology, promoting the spread out of cells and multinucleation. Interestingly, such changes did not interfere with the ability of lung epithelial cells to progress throughout the cell cycle without condemning cells to death, indicating the carcinogenic properties of these fibres. In fact, the uninterrupted proliferation of multinucleated cells inevitably leads to aneuploidy, which is largely known to participate to cancer development⁽¹⁸⁾.

The tendency of FE fibres to act as a transforming agent was also supported by the ability of treated epithelial cells to produce pro-inflammatory cytokines. It was shown that fibrous FE is able to promote the secretion of Interleukin 6 (IL-6), a multifunctional cytokine with immuno-regulatory and pro-inflammatory effects, and IL-8, a potent chemo-attractant for polymorphonuclear leuko-

cytes, from lung epithelial cells⁽¹⁷⁾. In fact, surviving cells that continue to release IL-6 and IL-8, could trigger a chronic inflammatory process, a phenomenon known to be tightly related to many types of cancer^(19,20). In 2007, Cardile et al.⁽²¹⁾ conducted the study aimed to determine comparatively biological responses of human non-malignant mesothelial (Met-5A) and J774 cells following exposure to two types of FE fibres having low and high iron content. The study demonstrated that both forms of FE induced the Heat Shock Protein 70 (HSP70), ROS and RNS generation and decreased cell viability. The J774 cells were more sensitive to FE in terms of nitrite biosynthesis than Met-5A cells.

The authors suggested, that the primary site of the fibre-induced inflammatory response could be on the macrophage rather than the pulmonary epithelium. They observed that high-iron fibres were more potent than low-iron fibres in stimulating ROS formation in both mesothelial and macrophage cells, while the low iron fibres were much more potent inducers of NOS in J744 cells than high iron fibres. Moreover the fibres increased expression of Hsp70 protein levels in a concentration-dependent manner and it depends on ROS/RNS levels, suggesting that extracellular Hsp70 may have a cytoprotective role at lower concentrations in response to exposure to mineral fibres in acute lung injury, but once a certain critical threshold is attained, it may potentiate the inflammatory response, subsequently resulting in significant auto-injury to the host⁽²¹⁾.

Critical parameters for tumour development, progression and survival such as cell motility, distribution of polymerized actin, vascular endothelial growth factor (VEGF) and β -catenin expression was investigated in the A549 and MeT-5A cell lines exposed to FE. Also the levels of cyclooxygenase (COX-2) and prostaglandin (PGE2), involved in cancer pathogenesis by affecting mitogenesis, cell adhesion, immune surveillance and apoptosis were investigated in J774 cell line, under the same experimental conditions. The study demonstrated that all these parameters were differently affected in both A549 and MeT-5A cell lines treated with FE. The increased levels of COX-2 and PGE-2 were also observed in J774 cell line, underlining the crucial role of the FE fibres in tumourigenesis⁽²²⁾.

Among the *in vitro* studies also the expression of retinoblastoma protein (Rb), which plays an important role in cell cycle and tumour progression, related to the exposure to FE fibres, was studied

(Fig. 4). The study was conducted on A549 and MeT-5A cell lines, which exposed to high concentrations of FE fibres determined the overexpression of phosphorylated retinoblastoma protein (pRb), but did not exhibit changes in Rb expression. These findings suggest a role for pRb as a cell cycle regulator and promoter of cell proliferation, that contribute to lung tumourigenicity related to FE fibre exposure⁽²³⁾. Also the role of phosphoinositide C (PLC), which is implicated in the control of cell growth and differentiation, was analysed in A549 cell line exposed to FE. The increased expression of two isoforms of PLC (PLC β 1 and PLC γ 1) was observed⁽²⁴⁾, confirming again that the exposure to FE fibres is implicated in cancer development and progression (Table 1).

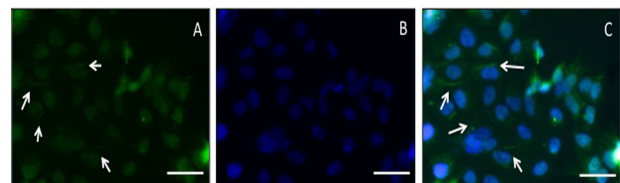


Fig. 4: Immunocytochemistry Analyses in MeT-5A cells exposed to fluoro-edenite fibres.

A). FITC-labelled antibody (green staining) used to visualize immunocytochemical retinoblastoma protein expression in MeT-5A cells exposed to 100 μ g/ml fluoro-edenite fibres. Fluoro edenite fibres (white arrows). **B).** DAPI (blue staining) used to visualize immunocytochemical retinoblastoma protein expression in MeT-5A cells exposed to 100 μ g/ml fluoro-edenite fibres. **C).** FITC and DAPI staining used to visualize immunocytochemical retinoblastoma protein expression in MeT-5A cells exposed to 100 μ g/ml fluoro-edenite fibres. Fluoro edenite fibres (white arrows). **A-C.** Color of positive staining was defined as the presence of a fluorescence detection on the edge of the black background. Cytoplasmic or membrane staining by FITC (green fluorescence) and nucleus staining by DAPI (blue staining). Magnification X40; scale bars: 50 μ m.

Retrospective analysis of the *in vivo* studies

To go deeper into this matter, several epidemiological and environmental studies have been undertaken to test the hypothesis of a causal association of mesothelioma onset with exposure to FE fibres. Preliminary results obtained in a sheep population living in the Biancavilla area show that FE fibres could be detected in the pulmonary parenchyma of nearly 30% of the examined sheep⁽²⁵⁾, suggesting a possible bio-indicative role of sheep as sentinel animals in the evaluation of environmental

fibre diffusion. A similar study was reported also by Rapisarda et al. in 2005⁽²⁶⁾, who measured the concentration of FE fibres in the lymph nodes draining the lung lobes of sheep habitually grazing 3 km from Biancavilla, and they reported that FE fibres in lymph nodes appear to be a helpful tool to conduct its environmental monitoring.

These findings support the idea of the bio-persistence of FE fibres in vivo, supporting the validity of the short-term studies conducted previously in vitro. Sheep lung is comparable in architecture, volume and respiratory physiological parameters to human lung⁽²⁷⁾, and thus it can be used to elucidate the pathophysiology related to exposure to mineral fibres in lung diseases⁽⁷⁾. The first in vivo study related to the exposure to FE, using the sheep model, was carried out in 2006 by Martinez et al.⁽²⁸⁾, who investigated the pathological effects of the inhalation of FE fibres, related to apoptotic processes and histological changes of lung architecture through the expression of TNF-related apoptosis-inducing ligand (TRAIL) and its receptor DR5, and of matrix metalloproteinase protein (MMP-13) involved in the breakdown of extracellular matrix. The authors observed the overexpression of the MMP-13, particularly in fibroblasts and epithelial cells, suggesting its involvement in the loss of the lung architecture.

In fact, they noted the loss of alveolar architecture with honeycombing of alveolar cavities, and the hyaline degeneration and proteoglycans alterations in the parenchyma, which could be associated with the fibrosis exhibited by the sheep lung samples. Moreover, the expression of TRAIL and its receptor was observed in areas of inflammatory infiltration and active fibrosis (alveolar surfaces), which confirmed the link between FE and epithelial cell apoptosis at the site of initial fibre deposition in the bronchioalveolar duct region. Further in vivo study on the lung tissue from sheep, carried out by Loreto et al.⁽²⁹⁾ in 2008, detected the immunohistochemical localization of bcl-2 and bax, respectively anti- and pro-apoptotic proteins. Low bcl-2 immunoreactivity and bax up-regulation was observed, indicating that apoptosis is an important mechanism for removing cells with irreparable FE-induced genetic changes that predispose them to a neoplastic evolution.

Another in vivo study using an in vivo sheep model was performed in 2010 by Musumeci et al.⁽³⁰⁾, who observed the overexpression of the phosphorylated retinoblastoma protein (pRb) in alveolar

epithelium and interstitium, close to the FE fibres, suggesting that the up-regulation of pRb could be a programmed response to protect the organism against uncontrolled cell proliferation (Fig. 5 and 6). In a recent and interesting study, for the first time pleural plaques have been detected in the lungs of subjects both residents and workers in Biancavilla (Catania, Sicily, Italy) not exposed to asbestos but to fluoro-edenite fibres. This finding suggests that pleural plaques can be ascribed to exposure to this amphibole fibre and highlight even more the correlation between the onset of MPM and exposure to FE fibres⁽³¹⁾ (Table 1).

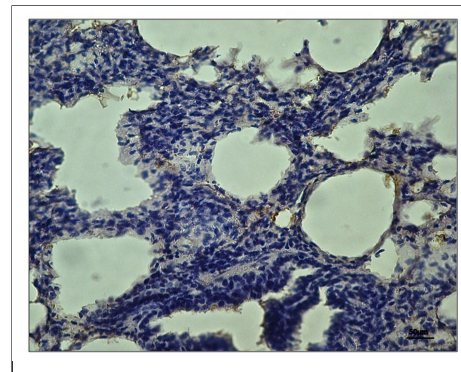


Fig. 5: Immunohistochemistry analyses. Alveolar and interstitial immunolabeling of phosphorylated retinoblastoma protein in unexposed sheep lung tissue. Magnification X40; scale bar: 50 μ m.

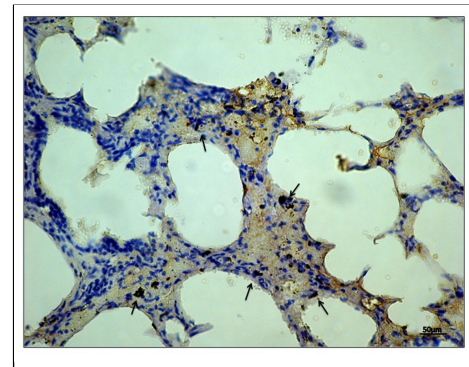


Fig. 6: Immunohistochemistry analyses. Phosphorylated retinoblastoma protein immunorepression in exposed (fluoro edenite fibres) sheep lung tissue. Note, besides the immunonegativity, the fluoro-edenite fibres in close contact with lung alveolar epithelium and in the interstitium (black arrows). Magnification X40; scale bar: 50 μ m.

Main pathogenic factors correlating FE to MPM

What has been observed until today, for what regards the relationship between the exposure to FE fibres and the development of MPM, is that the

pathogenesis of this tumour is principally based on the oxidative stress and inflammatory process. It is widely known that the chronic inflammation is associated with cancer development⁽³²⁾. The most important cells implicated in the tumour pathogenesis are the macrophages, which constitute a major component of the infiltrate of the most, if not all, tumours⁽³³⁾. Many tumour cells produce cytokines called colony-stimulating factors that prolong survival of macrophages. When appropriately activated, the latter can kill tumour cells or elicit tissue destructive reactions centred on the vascular endothelium. However, macrophages also produce growth and angiogenic factors as well as protease enzymes which degrade the extracellular matrix. Hence, macrophages can stimulate tumour-cell proliferation, promote angiogenesis, and favour invasion and metastasis⁽³⁴⁾. It was observed that the FE exposure is associated with the increased production of pro-inflammatory cytokines, such as IL-6 and IL-8⁽¹⁸⁾, COX-2 and PGE-2⁽²³⁾, implicated in the inflammatory process and of the VEGF⁽²³⁾ and MMP-13⁽²⁹⁾, the first associated with the promotion of angiogenesis and the second with degradation of the extracellular matrix. Taking into account what has been said before, these findings suggest the fundamental role of the exposure to FE in the pathogenesis of MPM.

Moreover, FE has been also associated with the increased oxidative stress, related to the mediation of inflammatory fibrosis of the lung and DNA damage, which finally and probably results in lung carcinoma and mesothelioma^(14-16,22). Condition of oxidative stress has been related to the conversion of NO, which levels increase in cells exposed to FE fibres⁽¹⁷⁾, to peroxy nitrite, a free radical form with cyto and genotoxic features⁽³⁵⁾ and with the overexpression of Hsp70, an important regulator of cancer cell growth, which is able to potentiate the inflammatory response^(17,36). It was also observed that the toxicity linked to the increased production of ROS and RNS is stronger in the inflammatory disorders, which are considered, thus, capable to increase the risk of lung cancer⁽¹⁷⁾.

Moreover, it has been suggested that oxidative stress may play a critical role in MPM carcinogenesis by promoting epithelial to mesenchymal cell transition processes and enhancing the expression of stemness genes⁽³⁷⁾. It has been described then, that FE fibres induced aberrant cell motility, distribution of polymerized actin and expression β -catenin, underlying again their involvement in can-

cer pathogenesis⁽²³⁾. Implication of FE fibres in tumourigenesis, tumour progression and its survival was also highlighted by the overexpression of cell cycle regulators pRb^(24,31) and PLC⁽²⁵⁾. Exposure to FE fibres is also responsible of changes in the lung epithelial cells morphology. In fact, they promote the spread out of cells and multinucleation without interfere with the ability of these cells to progress throughout the cell cycle, without condemning cells to death and leading to aneuploidy, which participates to cancer development⁽¹⁹⁾. Moreover, the loss of alveolar architecture with honeycombing of alveolar cavities, and the hyaline degeneration and proteoglycans alterations in the parenchyma, which could be associated with the fibrosis, was noted⁽²⁹⁾.

Conclusions

Different insights exist about the reaction of the lung cells to the exposure to FE fibres. Several studies confirm their analogy with the asbestos fibres, as well as their possible implication in the onset and progression of the MPM. Anyway, how the cellular events converge in the pathogenesis of MPM remains enigmatic. Understanding of how pleural mesothelial cells respond to FE fibres might be helpful to design preventive approaches to protect population from consequences of exposure to this airborne mineral fibre and may have important implications for the therapeutic strategies.

Abbreviation list

COX-2, cyclooxygenase; CT, Computed Tomography; FE, Fluoro-Edenite; HSP70, Heat Shock Protein 70; IL, Interleukin; LDH, lactate dehydrogenase; LPS, Lipopolysaccharides; MMP-13, matrix metalloproteinase protein; MPM, Malignant Pleural Mesothelioma; MRI, Magnetic Resonance Imaging; NO, nitric oxide; PET-CT, Positron Emission Tomography-CT; PGE2, prostaglandin; PLC, phosphoinositide C; pRb, phosphorylated retinoblastoma protein; Rb, retinoblastoma protein; RNS, nitrite species; ROS, reactive oxygen; TRAIL, TNF-related apoptosis-inducing ligand; US, ultrasound; VEGF, vascular endothelial growth factor; WTI, Wilm'tumour gene-1.

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