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Role of prostaglandin E2 in the invasiveness, growth and protection of cancer cells in malignant pleuritis

Elisabetta Pace[a,], Liboria Siena[a], Maria Ferraro[a], Mirella Profita[a], Patrizia Mondello[b], Giuseppina Chiappara[a], Angela Marina Montalbano[a], Antonino Giarratano[c], Giovanni Bonsignore[a], Mark Gjomarkaj[a]*

[a]*Istituto di Biomedicina e Immunologia Molecolare, Consiglio Nazionale delle Ricerche, Via Ugo La Malfa, 153, 90146 Palermo, Italy*
[b]*Azienda Ospedaliera V. Cervello, Palermo, Italy*
[c]*Dipartimento di Anestesiologia, Rianimazione e delle Emergenze, Università degli Studi di Palermo, Palermo, Italy*

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ABSTRACT

The recurrence of pleural effusions is a common event in a variety of neoplastic diseases. The objective of this study was to identify the mechanisms promoting the homing and growth of cancer cells within the pleural space. A cancer cell line recovered from malignant pleural fluids (lung adenocarcinoma cell line) that constitutively expresses cyclooxygenase 2 (COX-2) and all types of prostaglandin receptors was studied. It was first demonstrated using a matrigel system, that malignant pleural fluids increase the invasiveness of adenocarcinoma cells more than congestive heart failure (CHF) pleural fluids. Moreover, exposure to exudative malignant, but not to CHF pleural fluids, increased the mRNA (measured by real-time polymerase chain reaction (PCR)) and protein expression of COX-2 (measured by Western blot), as well as the activation and nuclear translocation of nuclear factor κB (NFκB) in cancer cells. These events are all actively regulated by prostaglandin E2 (PGE2), since the addition of synthetic PGE2 to cancer cells and the depletion of PGE2 from malignant pleural fluids or the inhibition of COX-2 activity significantly increased and reduced these phenomena, respectively. Moreover, malignant pleural effusions and synthetic PGE2 increased the long-term proliferation of cancer cells and reverted the impairment in long-term proliferation due to talc exposure. This study demonstrates that PGE2 present in malignant effusions contributes to cancer expansion and may protect cancer cells by anti-proliferative effects induced by talc.

1. Introduction

Malignant pleural effusions recur in 15% of patients who die with malignancies, account for up to 50% of the exudates in many clinical series,[1] and are related to a primary tumour (mesothelioma) or, more frequently, to metastatic adenocarcinoma tumours of lung, ovarian, gastrointestinal or breast origin. Tumour invasion of the pleural compartment activates multiple biological mechanisms, which result in the accumulation of fluid enriched in proteins in the pleural space (i.e. the development of an exudative pleural effusion).[1,2] Although the recurrence of a malignant pleural effusion is a common event, the mechanisms of tumour localisation into the pleura, as well as the mechanisms propelling the growth of metastases within the pleura, are not well elucidated.[1]

* *Corresponding author:* Tel.: +39 091 680 9148; fax: +39 091 680 9122.
E-mail address: pace@ibim.cnr.it (E. Pace).
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Recent studies have further elucidated the long-recognised relationship between the pathological processes of infection, inflammation and cancer.[3] Inflammation promotes the first stage of neoplastic transformation, also known as 'initiation', as well as tumour growth, by stimulating cell proliferation, adhesion, vascularisation and by increasing the metastatic potential of later stage tumours. However, little is known about the contribution of inflammation to the 'homing' of cancer cells in particular metastatic sites and to the growth of metastatic cells in the new compartments.

Previous studies, concerning a variety of tumours, have implicated the expression of cyclooxygenase 2 (COX-2) in different steps of tumour genesis, including tumour invasiveness[4] and cancer cell proliferation and resistance to apoptosis.[5,6] One of the best-known and most well-studied metabolites due to COX-2 activation is prostaglandin E2 (PGE2).[5] PGE2 is produced by a variety of cells, including macrophages and some types of malignant cells, and exerts its activities close to the site of production by binding to one or to a combination of four subtypes of receptor (EP1, EP2, EP3 and EP4).[5] This mediator regulates immunity and inflammation and plays an emerging and crucial role in cancer progression. The inhibition of COX-2 in human head and neck cancer results in loss of intra-tumour PGE2 levels and in turn leads to a reduced proliferation and to increased apoptosis of cancer cells.[7] Moreover, PGE2 inhibits cytotoxic activity of monocytes against cancer cells by decreasing the release of tumour necrosis factor-α (TNF-α).[8]

The present study was performed: (i) to assess whether malignant pleural fluids contain soluble factors able to promote the 'homing' of lung adenocarcinoma cancer cells within the pleural space; (ii) to address whether malignant pleural fluids contain soluble factors able to increase COX-2 expression in cancer cells within the pleural space; (iii) to evaluate the contribution of PGE2 to cancer invasiveness and cancer growth within the pleural space. The results show that the release of PGE2 during pleural malignant inflammation actively contributes to cancer 'homing' as well as to cancer growth and protection within the pleural compartment.

2. Material and methods

2.1. Pleural fluid collection

Pleural fluids were collected from patients with congestive heart failure (CHF) ($n = 6$, age range 50–78 years) and cancer ($n = 6$, age range 41–75 years). All subjects gave informed written consent and the study was approved by the institutional review board for human studies. The effusions were first classified as transudates or exudates by meeting at least one of the criteria described by Light.[9] CHF effusions were defined as transudates associated with an enlarged heart, distended neck veins and a cardiac gallop that improved with therapy for the CHF. Malignant effusions were defined as exudates associated with a pathological diagnosis of cancer from cytological examination of pleural fluids and from pathological examination of lung tissues. No patients were undergoing anti-inflammatory or steroid therapies. The fluids were drawn into polypropylene bags containing heparin (10–20 IU/ml). Cell-free fluids were frozen immediately at −70 °C until they were used in subsequent experiments.

2.2. Cancer cell lines

Human pleural metastatic adenocarcinoma (Colo699N) and human mesothelioma (MSTO-211) cells, were purchased from the Interlab Cell line Collection (Genova, Italy) and from the American Type Culture Collection (American Type Culture Collection, Rockville, MD, United States of America (USA)), respectively. Cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated (56 °C, 30 min) foetal bovine serum, 2 mM glutamine, 1 mM sodium pyruvate and 1% penicillin-streptomycin solution (all from GIBCO, Grand Island, NY, USA). Cells were stimulated with pleural fluids from cancer and from CHF patients, with PGE2-depleted malignant pleural fluids and, in other experiments, with PGE2 (1 ng/ml) (Sigma, Aldrich, Milan, Italy) and with talc (50 μg/cm^2) (Sigma) for 24 h.[10] Optimal concentrations of PGE2 and talc were assessed by preliminary experiments (data not shown). The experimental setting also included the use of a specific inhibitor of COX-2 (SC58236) (10 μM) (Searle Corporation, St. Louis, MO, USA).

2.3. Invasiveness of cancer cells

The invasiveness of adenocarcinoma cells was assessed using matrigel-coated invasion chambers (Becton Dickinson Labware, Franklin Lakes, NJ, USA) as described previously.[11] Briefly, cell-free pleural fluids from CHF and cancer patients were incubated in the lower chamber and the adenocarcinoma cells (5×10^4) in the upper chamber for 18 h at 37 °C in a humidified 5% CO_2 atmosphere. The cancer cells on the upper surface of the matrigel were then removed, fixed with methanol and the membranes stained with haematoxylin/eosin, and the cells adherent to the outer surface of membrane evaluated, counting at least six fields per filter in each group at 400 × magnification.

2.4. Depletion of PGE2

Pleural fluids were depleted from PGE2 with a commercially available PGE2 affinity sorbent (mouse anti-PGE2 IgG covalently bound to Sepharose 4B) (Cayman Chemical, Ann Arbor, MI, USA) as described previously.[12] The concentrations of PGE2, following PGE2 affinity sorbent, were below the lower sensitivity limit of the enzyme-linked immunosorbent assay (ELISA) kit (Amersham Biosciences, UK).

2.5. Real-time polymerase chain reaction (PCR) analysis of COX-2 by cancer cells

Total cellular RNA, extracted as described previously[13], using RNAzol kit (Biotech Italia, Rome, Italy), was reverse-transcribed to cDNA, using M-MLV-RT and oligo(dT)$_{12-18}$ primer (Invitrogen, Carlsbad, CA, USA). Real-time quantitative PCR of human COX-2 gene was carried out on ABI PRISM 7900 HT Sequence Detection Systems (Applied Biosystems, Foster City, CA, USA)[14] using specific FAM-labelled probe and primers (Applied Biosystems, TaqMan Assays on Demand).

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene expression was used as endogenous control for normalisation. Relative quantitation of mRNA was carried out with a comparative computed tomography (CT) method as described previously.[15]

2.6. Expression of COX-2 and of EP receptors by cancer cells

The expression of COX-2 protein and of EP receptors were evaluated by Western blot analysis, as described previously,[16] using rabbit polyclonal antibodies (Cayman Chemicals) specific for COX-1, COX-2, EP1, EP2, EP3 and EP4 receptors. The absence of primary antibody or isotype matched control antibodies were utilised for negative controls. Beta-actin (Sigma) was used as housekeeping protein.

2.7. Expression and activation of NFκB by cancer cells

To study the expression and the nuclear translocation of nuclear factor κB (NFκB), the cytoplasmic and nuclear protein fractions were separated by using a commercial kit (Pierce, Rockford, IL, USA). Western blot analyses were performed on separated protein fractions, as described above, using a polyclonal antibody recognising subunit p65 of NFκB (Santa Cruz Biotechnologies, Santa Cruz, CA, USA). A commercially available ELISA kit was used (SuperArray Bioscience, Frederick, MD, USA) to assess NFκB activation.

2.8. Clonogenic assay of cancer cells

The colony growth of cancer cells (adenocarcinoma and mesothelioma cells) cultured with pleural fluids depleted and not depleted from PGE2, with PGE2 and with talc was evaluated as described previously.[17] Each experiment was conducted in triplicate. At the end of the incubation, colonies (aggregates with at least 40 cells) were counted under an inverted phase-contrast microscope (Leitz, Wetzlar, Germany).

2.9. Statistics

Data are expressed as mean counts ± standard deviation. Analysis of variance (ANOVA) corrected with the Bonferroni test and *t*-test were used for comparisons. $P < 0.05$ was accepted as statistically significant.

3. Results

3.1. Malignant pleural fluids attract cancer cells

Since the pleura is frequently involved in metastatic neoplastic processes, we first assessed whether pleural fluids from lung cancer patients contained soluble factors promoting the homing of cancer cells within the pleural space. A lung adenocarcinoma cell line was incubated with and without pleural fluids from cancer patients and from CHF patients and the invasiveness of cancer cells was evaluated on the basis of their ability to digest matrigel. Surprisingly, when cancer cells were cultured in the presence of malignant pleural fluids, but not in the presence of CHF pleural fluids, they significantly and dramatically increased their ability to digest matrigel. To assess the contribution of pleural PGE2 to the increased invasiveness of adenocarcinoma cells, matrigel experiments were performed with native pleural fluids and with PGE2_ depleted pleural fluids and synthetic PGE2. Interestingly, the depletion of PGE2 from malignant pleural effusions significantly reduced the ability of cancer cells to digest matrigel. The presence of synthetic PGE2 increased the ability of cancer cells to digest matrigel (Fig. 1).

3.2. Malignant pleural fluids upregulate COX-2 expression on metastatic and primitive pleural cancer cells

Since the upregulation of COX-2 in cancer cells is associated with the increase in the invasiveness of cancer cells,[4] we determined whether the presence of malignant pleural fluids was able to affect the expression of COX-2 mRNA in adenocarcinoma cells. The presence of malignant pleural fluids, but not of transudative pleural fluids, significantly upregulated COX-2 mRNA expression in the tested cell line in comparison with baseline (Fig. 2(a)). It was then assessed whether PGE2 present in malignant pleural fluids contributed to the upregulation of COX-2 mRNA exerted by pleural fluids. The deprivation of PGE2 from malignant pleural fluids significantly decreased the COX-2 mRNA expression of cancer cells (Fig. 2(a)), suggesting that PGE2 present in malignant pleural fluids actively contributes, in an autocrine manner, to the increased expression of COX-2 in cancer cells. Moreover, we assessed whether the increase in COX-2 mRNA paralleled the increase in COX-2 protein. The presence of malignant pleural

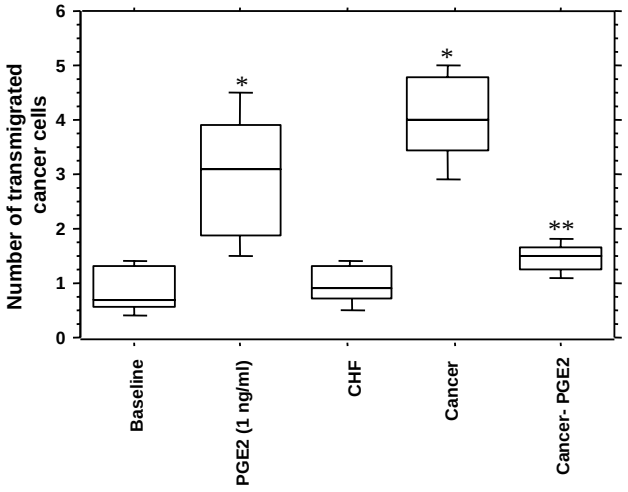

Fig. 1 – Pleural prostaglandin E2 (PGE2) present in malignant pleural fluids attract cancer cells. Adenocarcinoma cells were cultured in the presence and in the absence of exudative (from cancer patients; *n* = 6) and transudative (from congestive heart failure (CHF) patients; *n* = 6) pleural fluids for 24 h and were used for matrigel matrix assay. Exudative pleural fluids depleted from PGE2 (see Material and methods section for details) and synthetic PGE2 (1 ng/ml) were also included. Results are expressed as mean ± SE. *P < 0.05 compared with the baseline; **P < 0.05 compared with cancer.

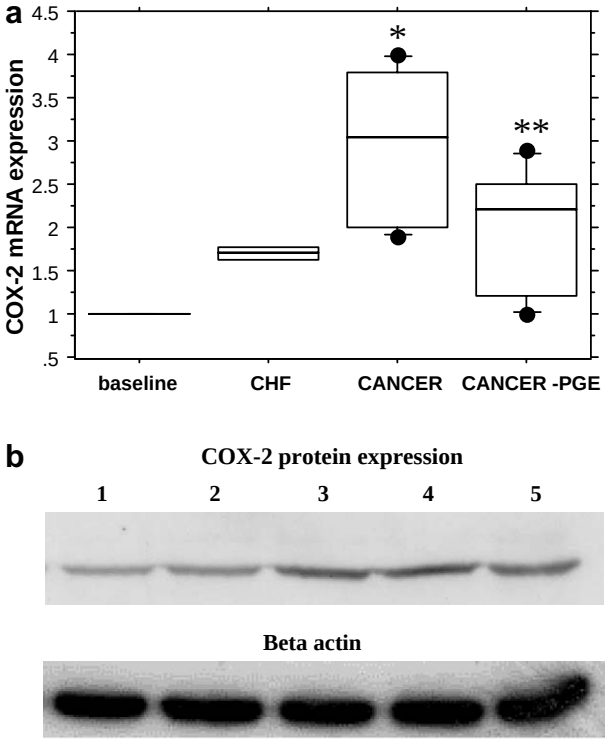

Fig. 2 – Malignant pleural fluids upregulate cyclooxygenase 2 (COX-2) expression on metastatic and primitive pleural cancer cells. Adenocarcinoma cells were cultured for 24 h in the presence and in the absence of exudative (n = 6) pleural fluids, depleted and not depleted for prostaglandin E2 (PGE2) (see Material and methods section for details), and of transudative (n = 3) pleural fluids and were used for evaluating COX-2 mRNA and protein expression. (a) COX-2 mRNA expression was quantified by real-time reverse transcription-polymerase chain reaction (PCR) assays. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RNA was used for normalisation. Data are expressed as fold induction over control untreated samples. $^{*}P < 0.05$ compared with the baseline. $^{**}P < 0.05$ compared with cancer; (b) representative Western blot showing the expression of COX-2 protein at baseline level (lane1) and following the exposure to a transudative (lane 2) and to three different exudative pleural fluids (lanes 3, 4, 5). Membranes were then stripped and incubated with goat polyclonal anti-β-actin.

fluids was also able to increase the COX-2 expression at a protein level (Fig. 2(b)), but it was not able to increase COX-1 expression (data not shown). All these events were not related to the upregulation of specific EP receptors since the constitutive expression of all four types of EP receptors was not affected by malignant pleural effusions or by synthetic PGE2 (data not shown).

3.3. *Malignant pleural fluids upregulate NFκB expression and activation*

Since it is well known that the activation of COX-2 is frequently associated with the upregulation of nuclear factor kB (NFκB),[18,19] the activation and nuclear translocation of NFκB in lung cancer cells was evaluated. Interestingly, the presence of malignant pleural fluids upregulated both the activation (Fig. 3(a)) and the nuclear translocation (Fig. 3(b)) of NFκB. The depletion of PGE2 and the presence of a specific COX-2 inhibitor significantly reverted these effects, suggesting that the presence of PGE2 contributes to the increased activation and nuclear translocation of NFκB due to malignant pleural fluids via COX-2 activation (Fig. 3(a and b)).

3.4. *Malignant pleural fluids promote cancer cell proliferation*

We used a clonogenic assay to explore whether malignant pleural fluids contained soluble factors affecting the self-renewal capacity of cancer cells. Surprisingly, malignant

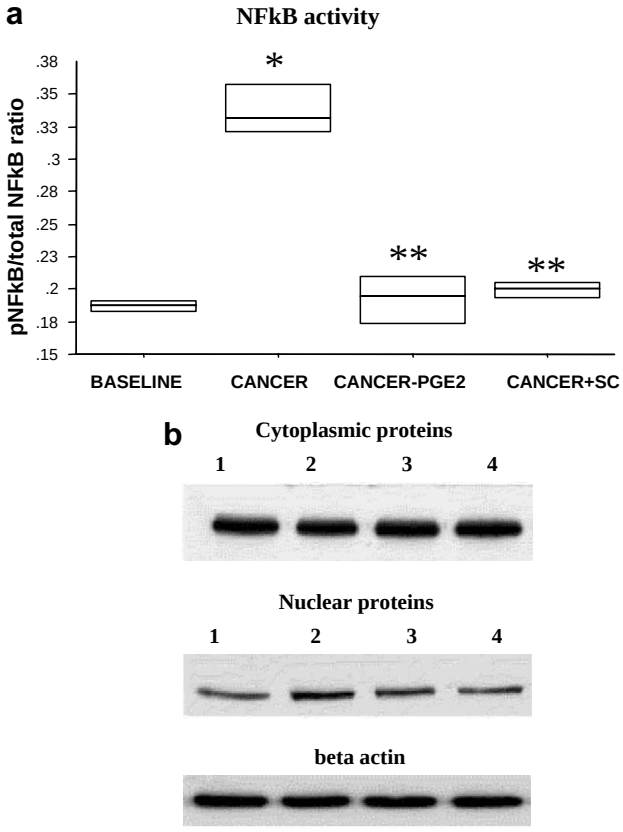

Fig. 3 – Malignant pleural fluids upregulate nuclear factor κB (NFκB) expression and activation. Adenocarcinoma cells were cultured for 3 h with exudative (from cancer patients; n = 6) pleural fluids depleted and not depleted for prostaglandin E2 (PGE2) and with pre-incubation with a specific inhibitor (SC58236) (10 μM) of cyclooxygenase 2 (COX-2) activity. (a) NFκB activity was evaluated using an enzyme-linked immunosorbent assay (ELISA) kit. Data are expressed as phosphorylated NFκB (pNFκB)/total NFκB ratio. $^{*}P < 0.05$ compared with the baseline. $^{**}P < 0.05$ compared with cancer. (b) Representative Western blot performed on cytoplasmic and nuclear extracts. Lane 1 = baseline; lane 2 = cancer; lane 3 = cancer without PGE2; lane 4 = cancer + SC58236.

pleural fluids significantly increased the numbers of cancer colonies, while the deprivation of PGE2 from malignant pleural fluids or the addition of a specific COX-2 inhibitor abrogated this effect (Fig. 4), suggesting that the activation of COX-2 pathway and PGE2 present in malignant pleural fluids actively contribute to promoting the growth of cancer cells within the pleural space.

3.5. *Paracrine PGE2 protects cancer cells*

It was also determined whether PGE2 or talc, the latter an agent commonly used in pleurodesis procedures, may affect the clonogenic activity of cancer cells. The same effects were also tested on a mesothelioma cell line chosen as an example of primary pleural cancer cells. While talc dramatically and significantly reduced the colony formation, PGE2 was able significantly to increase the colony formation in both the studied cell lines (Fig. 5(a and b)). It was also explored whether PGE2 may act as protective agent for cancer cells, by co-culturing cancer cells with talc and PGE2. Surprisingly, the addition of PGE2 was able to completely revert the activity of talc in reducing the colony formation in both the studied cell lines (Fig. 5(a and b)).

3.6. *Upregulation of COX-2 protects cancer cells*

Finally, experiments were performed to evaluate whether the protective effects of PGE2 on adenocarcinoma and mesothelioma cells were associated with an increase in COX-2 expression, at both mRNA and protein levels. Interestingly, PGE2 was able to upregulate the expression of COX-2 at mRNA and at protein levels (Fig. 6(a and b)) in both the tested cell lines. Surprisingly, also talc was able to upregulate COX-2 (mRNA and protein) expression. The combined addition of talc and PGE2 further increased the expression of COX-2 (Fig. 6(a and b)) in the tested cell lines.

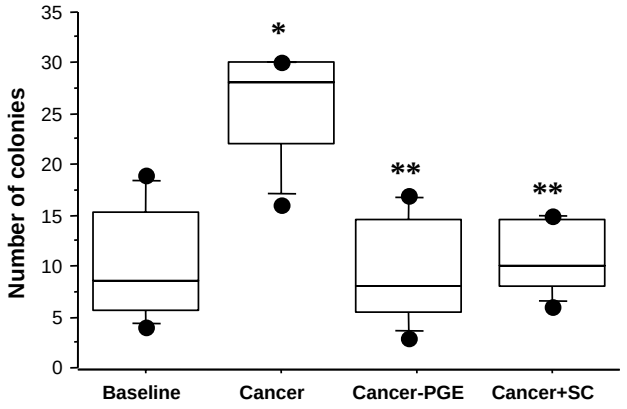

Fig. 4 – Malignant pleural fluids promote cancer cell proliferation. Cancer cells were cultured in the presence and absence of exudative pleural fluids (from cancer patients; *n* = 6) depleted and not depleted for prostaglandin E2 (PGE2) and were used for evaluating the long-term proliferation of cancer cells using a clonogenic assay (see Material and methods for details). Data are expressed as mean ± SE number of colonies. *P < 0.05 compared with baseline. **P < 0.05 compared with cancer.

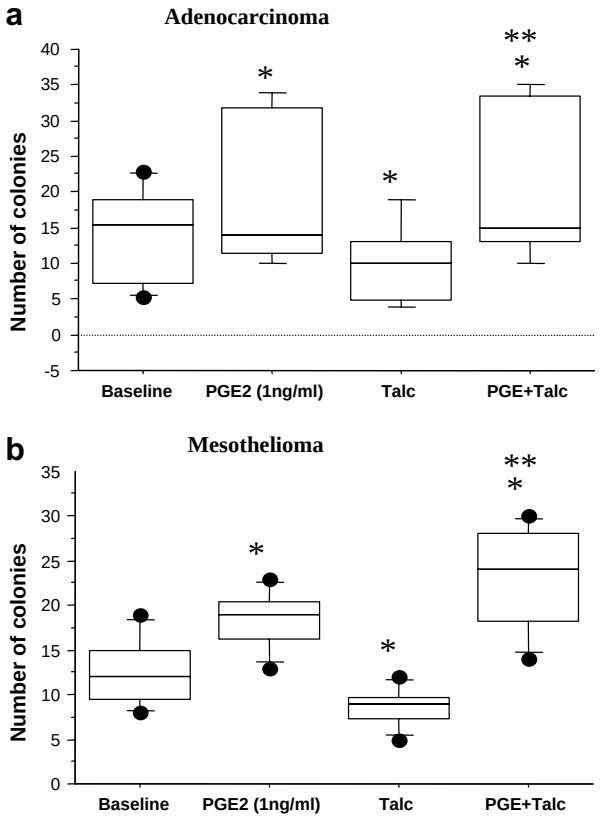

Fig. 5 – Prostaglandin E2 (PGE2) protects cancer cells from the anti-proliferative effects of talc. (a) Adenocarcinoma and (b) mesothelioma cells were cultured in the presence and in the absence of synthetic PGE2 (1 ng/ml), of talc (50 μg/cm^2) and of combined talc and PGE2 for 24 h and were tested for their long-term proliferation using a clonogenic assay (see Material and methods for details). Data are expressed as mean ± SE of six independent experiments. *P < 0.05 compared with the baseline. **P < 0.05 compared with talc.

4. Discussion

The diagnosis of a malignant effusion signifies disease progression, and is associated with a worse prognosis regardless of the tumour site of origin.

Although it is well known that specific cancers, such as lung cancer or cancer of the breast, ovary, and stomach preferentially metastasise the pleural compartment,[1] the mechanisms that portend a predilection for the pleural compartment are not well elucidated.

This study demonstrates that pleural inflammation generates an ideal micro-environment to attract cancer cells, to promote their growth and their protection when exposed to toxic agents. PGE2 present in exudative pleural fluids contributes to all these events by upregulation of the expression of COX-2 and of the activation of NFκB by cancer cells. The biological events promoting the establishment of cancer cells in a metastatic site are multiple and very complex.[20] Certainly, within the pleural compartment, one of these events is represented by the adhesion of a malignant cell to the pleural mesothelium by the interaction between CD44 receptor and hyaluronic acid

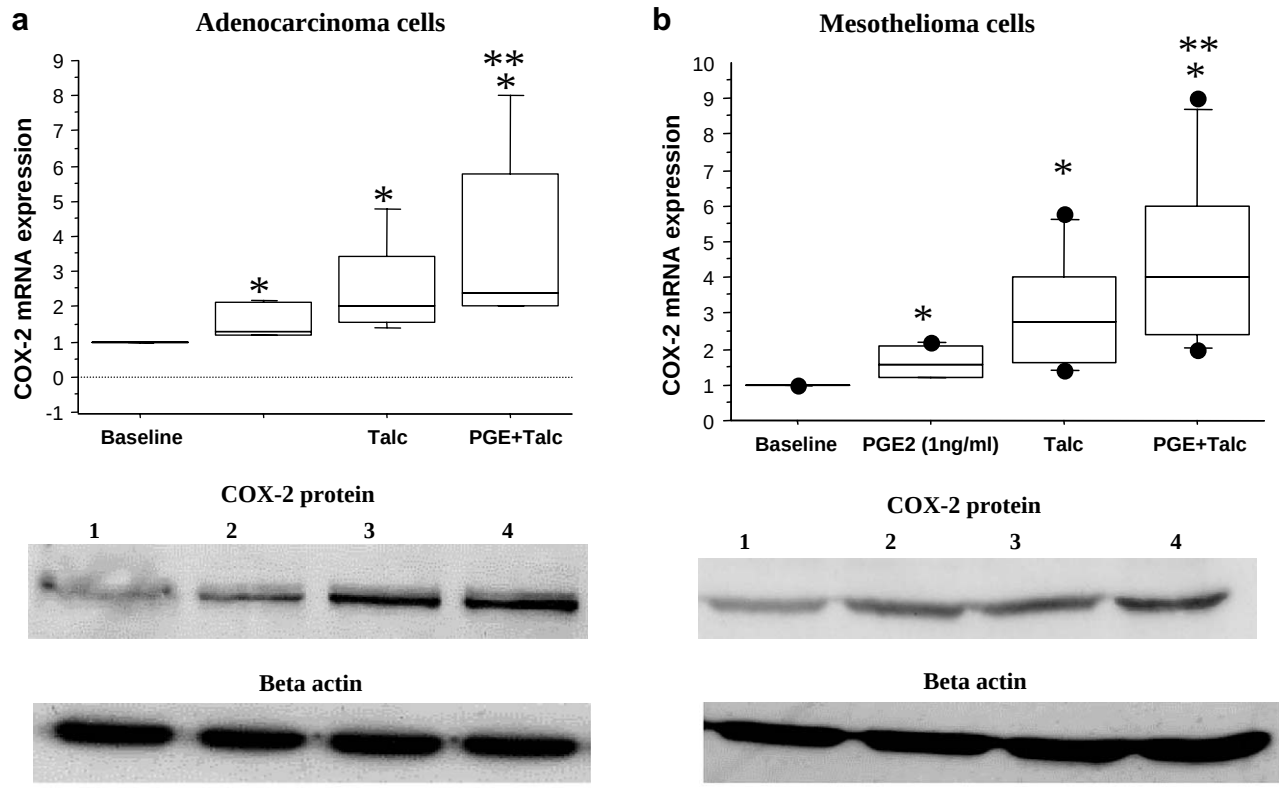

Fig. 6 – Upregulation of cyclooxygenase 2 (COX-2) protects cancer cells. Adenocarcinoma (a) and mesothelioma (b) cells were cultured in the presence and in the absence of synthetic prostaglandin E2 (PGE2) (1 ng/ml), of talc (50 μg/cm^2) and of combined talc and PGE2 and were used to assess COX-2 mRNA expression by real-time polymerase chain reaction (PCR) (see Material and methods for details). COX-2 mRNA was quantified by real-time reverse transcription-PCR assays. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RNA was used for normalisation. Data are expressed as fold induction over control untreated samples. Data are expressed as mean ± SE of five independent experiments. $^{*}P < 0.05$ compared with the baseline. $^{**}P < 0.05$ compared with talc. Representative Western blots showing the expression of COX-2 protein at baseline level (lane 1) and following the exposure to synthetic PGE2 (lane 2), to talc (lane 3), and to combined talc and PGE2 (lane 4). Membranes were then stripped and incubated with goat polyclonal anti-β-actin.

expressed by malignant cell and by mesothelial cells, respectively.[1] The elevated presence of hyaluronic acid on pleural mesothelium consequentially is one of the reasons why highly expressing CD44 cancers have a predilection for the pleura.[1] Data provided in this paper, may extend knowledge on why the pleura is a suitable metastatic site. In particular, our work evaluated the metastasis-promoting action exerted by pleural inflammation for the first time. It was demonstrated that malignant exudative pleural fluids contribute to implement the invasiveness of lung cancer cells. Exudative pleural fluids may act as chemo-attractant for cancer cells, since they increase the active movement of lung cancer cells and increase their ability to digest matrigel, a synthetic extracellular matrix. This phenomenon is associated specifically with the presence of an inflammatory milieu within the pleura since the transudative pleural fluids were not able to implement the invasiveness of cancer cells. This finding is very interesting, because it further underlines the association between inflammation and cancer.[21] We also provide information regarding the pleural inflammatory soluble mediator contributing to these phenomena. The finding that the depletion of PGE2 from the exudative pleural fluids significantly reduced the invasiveness of cancer cells suggests that PGE2 actively contributes to this phenomenon within the pleural space. Consistent with these findings, it has been demonstrated that the exposure of non-small cell lung cancer cells to PGE2 upregulates CD44, EP4, and matrix metalloproteinase 2 expression and enhances cancer cell invasion.[4] Further insight was obtained into the contribution of inflammation to these processes, by looking at the expression of COX-2, since it is known that COX-2 can affect cell invasion and since it has been demonstrated that higher COX-2 expression was observed in lung cancer lymph node metastasis than in primary adenocarcinoma.[6] A number of studies have shown a correlation between COX-2 expression and poor prognosis in non-small cell lung cancer.[6,22] It was demonstrated that, following exposure to exudative malignant, but not to CHF pleural fluids, metastatic cancer cells further increased their constitutive expression of COX-2 mRNA and protein, suggesting that pleural inflammation is able to generate a positive feedback loop to maintain high levels of COX-2 expression. This upregulation was promoted by the effect of PGE2 present in malignant effusions. Moreover, additional mechanisms, including the cross-talk with the inducible nitric oxide synthase/nitric oxide/cGMP pathway,

may account for this upregulation, as demonstrated in colorectal cancer.[23]

COX-2 and NFκB pathways are intimately related in human non-small cell lung carcinoma[19] and both may be considered hallmarks of inflammation.[21,24] The inhibition of COX-2 in human non-small cell lung carcinoma blocks the activation of NFκB due to exposure to cigarette smoke.[19] The NFκB transcription factor plays a key role in the induction of pro-inflammatory gene expression and also plays a variety of roles in lung cancer growth. The activation of NFκB in cancer cells promotes the resistance to chemotherapy, increases the expression of anti-apoptotic molecules and upregulates their ability to spread throughout the body.[21] On the basis of this evidence, we evaluated whether the presence of an inflammatory milieu was also able to increase the activation and nuclear translocation of NFκB. These phenomena were assessed by evaluating the pNFκB/total NFκB ratio and the expression of NFκB p65 subunit in cytoplasmic and nuclear protein fractions.[19,25] The presence of an inflammatory milieu increased both the activation and the nuclear translocation of NFκB, since an increase in the pNFκB/total NFκB ratio and in the intensity of the nuclear band were observed. Moreover, the relationship between NFκB and COX-2 pathways was further confirmed because it was shown that the presence of a specific COX-2 inhibitor reverted the activation and the nuclear translocation of NFκB associated with PGE2 present in inflammatory pleural fluids.

Tumour growth is dependent on the balance between increased proliferation and decreased cell death. A number of studies have demonstrated the role of COX-2 in this process.[6] Increased COX-2 expression is also associated with increased levels of downstream enzymes required for prostanoid synthesis, including prostaglandin E2 synthase (PGE-S). The release of high levels of PGE2 can, in turn, promote cellular survival as well as cancer cell growth and invasion. In this regard it has been demonstrated recently that PGE2 is able to rapidly induce Erk-mediated lung cancer cell proliferation.[26] Moreover, forced COX-2 expression in normal intestinal epithelial cells results in increased bcl-2 expression[27] and leads to a prolongation in the cell cycle G_1 phase.[28] Our observation that malignant exudative pleural fluids upregulate two inflammatory pathways, such as COX-2 and NFκB, importantly involved in the control of tumour growth, prompted us to evaluate the effect of malignant pleural fluids on long-term proliferation of cancer cells. It was demonstrated that the addition of exudative malignant pleural fluids increased the long-term proliferation of metastatic cancer cells, as assessed by the increased number of cancer colonies. The addition of a specific COX-2 inhibitor abrogated this effect, demonstrating that the activation of this pathway actively contributes to promote the growth of cancer cells within the pleural space.

The presence of a pleural inflammatory fluid, rather than detrimental for the cancer cells, probably via the activation of both COX-2 and NFκB pathways, further promotes cancer cell growth. In this context, we have previously demonstrated that the presence of high levels of soluble ICAM-1, during pleural inflammation, inhibits adhesion between lymphokine activated killer cells and tumour cells.[29]

Finally, we performed experiments aimed to clarify whether the increase in the COX-2 pathway was associated with protective mechanisms in cancer cells. We extended these evaluations also to a primary pleural cancer cell line (mesothelioma cell line) to better understand whether these phenomena were limited to a metastatic cancer cell. In detail, we designed experiments in which we evaluated the effect of talc, alone and in combination with PGE2, on long-term proliferation and on COX-2 pathway. We selected talc linking to the clinical management of lung cancer patients with recurrent pleural effusion. The standard option for recurrent effusions is chemical pleurodesis, and sterilised talc is the best sclerosant agent.[1] Moreover, a previous report has demonstrated, in an *in vitro* model, the ability of talc to induce cell apoptosis in a mesothelioma cell line.[10] Here, it is demonstrated for the first time that talc may decrease the self-renewal capacity of adenocarcinoma as well as of mesothelioma cell lines. In addition, paracrine PGE2 protects cancer cells from the impairment in cell proliferation due to talc and this protection is associated with an increased COX-2 expression, at both mRNA and protein levels in both the tested cell lines. The finding that talc is also able to upregulate COX-2 may appear to be contradictory. Indeed, we evaluated COX-2 expression during the early events of cell activation (24 h) while colony formation is a biological test that evaluates the long-term effects (15–21 d) of cell activation. One possible explanation is that when cancer cells are exposed to an insult, there is an early and dual activation of deleterious and protective mechanisms. When the autocrine activation of some protective mechanisms, such as induction of COX-2, may be not sufficient to overcome the negative mechanisms, apoptosis or anti-proliferative effects are established in the long term. When the cancer cells are exposed to talc and paracrine PGE2, the further increase in the expression of COX-2 may promote the prevalence of the protective mechanisms. In this regard, a recent paper looking at COX-2 expression in lung cancer, reports that it is highly inducible by taxanes.[30] The over-activation of COX-2 may also contribute to cancer growth by impairing the host cell-mediated immunity mechanisms, which play an important role in the elimination of transformed cells. In this regard, it is known that PGE2 down-regulates the mRNA expression and the release of TNF-α by human blood monocytes[8] and that lung tumour-derived PGE2 simultaneously promotes the production of interleukin (IL)-10 and inhibits the production of IL-12 by macrophages.[31]

In conclusion, these data provide further and new molecular insights linking pleural inflammation with the promotion and growth of lung cancer metastases within the pleural compartment. These findings are clinically relevant, since they further support the utility of anti-inflammatory therapies for a complementary approach in treating lung cancer.

Conflict of interest statement

None declared.

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