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Paclitaxel downregulates tissue factor in cancer and host tumour-associated cells

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ABSTRACT

Paclitaxel, a microtubule-stabilising compound with potent anti-tumour activity, has been clinically used in a wide variety of malignancies. Tissue factor (TF) is often expressed by tumour-associated endothelial and inflammatory cells, as well as by cancer cells themselves, and it is considered a hallmark of cancer progression.

We investigated whether paclitaxel could modulate TF in human mononuclear (MN) cells, human umbilical vein endothelial cells (HUVEC) and the metastatic breast carcinoma cell line MDA-MB-231.

Cells were incubated with or without paclitaxel at 37 °C. At the end of incubation, cells were disrupted and tested for procoagulant activity by a one-stage clotting assay, for TF antigen levels by ELISA and TF mRNA by real-time RT-PCR. IL-6 and IL-1β were tested by ELISA in conditioned medium.

Both the strong TF activity and antigen constitutively expressed by MDA-MB-231 and the TF induced by LPS, TNF-α and IL-1β in MN cells and HUVEC were significantly reduced by paclitaxel. In the presence of paclitaxel, lower TF mRNA levels were also detected. Since paclitaxel has been shown to induce the expression of inflammatory genes in monocytes and tumour cells, we tested whether paclitaxel could influence IL-6 and IL-1β release from the cells used in this paper. Neither the constitutive expression of IL-6 and IL-1β by MDA-MB-231 nor the basal and LPS-induced release from MN cells and HUVEC was affected.

Our data support the hypothesis that the anti-tumour effects of paclitaxel may, at least in part, be mediated by the capacity of this drug to modulate the procoagulant potential of cancer and host cells.

1. Introduction

Paclitaxel, a plant-derived diterpene, is an anticancer drug that exerts antimitotic activities against a wide range of solid tumours.[1] The mechanism by which paclitaxel inhibits proliferation is related to its ability to stabilise microtubules by binding β-tubulin, consequently leading to mitotic arrest.[2] Although prevention of microtubule depolymerisation appears to be the key feature by which paclitaxel inhibits proliferation, pleiotropic effects exhibited by this drug may be responsible for its success where other chemotherapeutic agents, which also affect microtubule assembly, fail. Indeed,

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by focusing on gene induction studies, investigators have shown that paclitaxel modulates the expression of genes encoding membrane proteins, transcription factors, inflammatory cytokines and enzymes participating in cell proliferation and apoptosis with mechanisms independent of microtubule stabilisation.[3–7] Moreover, it has been shown that paclitaxel, at very low concentrations, exerts an antiangiogenic activity with no effect on microtubule assembly.[8]

Tissue factor (TF), the integral membrane glycoprotein which functions as a cellular receptor for plasma clotting factor VII/VIIa, is the major physiological trigger of the coagulation cascade.[9] In addition to its well established role in blood coagulation, TF has been shown to participate in a variety of processes linked to tumour biology, such as tumour growth, metastasis and angiogenesis.[10,11] Malignant cells constitutively express TF, and this expression has been shown to correlate to cell metastatic potential.[11–14] Although the mechanisms underlying TF participation in tumour progression are complex and still require full elucidation to be completely unravelled, both proteolytic activity of the TF-VIIa complex[15] and TF-induced intracellular signalling which may involve the engagement of the protease-activated receptors[16,17] activities have been reported. Moreover, tumour cells themselves can induce the expression of TF by host cells such as endothelial cells or monocyte/macrophages.[18–22] Thus, TF on tumour or host cell surface represents a major mediator of clotting activation at the tumour–host interface. In addition, in vascular cells around human breast cancer cells the *in situ* detection of TF correlated with the malignant phenotype, suggesting TF as a marker for the initiation of angiogenesis in cancer.[21] Thus, TF appears to play the role of an efficient bridge between thrombosis and cancer development in different settings: the so-called 'common soil' between cardiovascular disease and at least some types of cancers is presently a matter of deep investigation, and its pharmacological modulation is still an open problem.

In the present study, we investigated whether paclitaxel can modulate the expression of TF in the constitutively TF-expressing metastatic breast carcinoma cell line MDA-MB-231 and in host cells, namely human mononuclear (MN) cells and human umbilical vein endothelial cells (HUVEC).

Our results indicate for the first time that paclitaxel inhibits TF activity, antigen and mRNA expression in both MDA-MB-231 and stimulated host cells.

2. Materials and methods

2.1. Chemicals

Medium 199 (M199), Dulbecco's Modified Eagle's Medium (DMEM), RPMI 1640 Medium, sodium citrate tribasic solution 4%, lipopolysaccaride (LPS, from *Escherichia coli* 055:B5), dimethyl sulphoxide (DMSO), Trypan blue 0.4%, gelatin from porcine skin (type A), Paclitaxel from *Taxus Brevifolia*, endothelial cell growth factor (ECGF), gentamicin and tris buffered saline (TBS) obtained from Tris–OH were from Sigma Aldrich, St Louis, MO. Foetal bovine serum (FBS), penicillin/streptomycin, L-glutamine, Dulbecco's phosphate buffered saline (PBS) and 0.05% trypsin/0.02% EDTA in PBS were from Euroclone Life Sciences Division, Pero, (Italy). Fungizone (Amphotericin B) was from Zeneus Pharma Limited, Stevenage Herts, United Kingdom (UK). Calcium chloride was from S.A.L.F., Bergamo (Italy). Heparin was from Mayne Pharma s.r.l., Naples (Italia). Sodium chloride 0.9% was from Braun, Milan (Italy). Collagenase H (from *Clostridium Histolyticum*) was from Roche Diagnostics, Mannheim (Germany). Lymphoprep was from Axis-Shield PoC AS, Oslo (Norway). ELISA (Imubind TF Kit) was from Instrumentation Laboratory, Milano, (Italy). Human IL-6 and IL-1β immunoassays were from R&D Systems, Minneapolis, MN. Triton X-100 was from Fluka BiochemiKa, Buchs (Switzerland). Limulus assay was from Whittaker Bioproducts, Inc., Walkersvill, MD. The human brain thromboplastin standard was kindly donated by Dr. L. Poller, Manchester (UK). BCA protein assay kit was from Pierce Biotechnology, Rockford, IL. Recombinant human IL-1β and recombinant human TNF-α were from Peprotec, Rocky Hill, NJ. Tissue-culture dishes and multi-well plates were from Falcon Labware, Becton Dickinson, Franklin Lakes, NJ. Sterile pyrogen-free micro tubes were obtained from Sarstedt, Numbrecht (Germany). The monoclonal anti-TF antibody HTF1 was kindly donated by Dr. Yale Nemerson (Mt. Sinai School of Medicine, New York).

2.2. Cell isolation and culture

MDA-MB-231 (human, Caucasian, breast, from adenocarcinoma) cells were cultured in Dulbecco's Modified Eagle's Medium, supplemented with 10% FCS, 2 mM L-glutamine and 1X penicillin/streptomycin in a humidified atmosphere of 95% air/5% CO_2 incubator at 37 °C.

Endothelial cells were isolated from human umbilical cord vein by digestion with 0.5% collagenase as previously described[20]. For experiments, HUVEC were plated at a density of 8×10^4 cells/well in gelatin-coated 12-well plates, and were grown to confluency in DMEM, supplemented with 15% FBS, 2 mM L-glutamine 100 μg/ml streptomycin, 100 U/ml penicillin, 1X ECGF and 10 mg/ml heparin in a humidified atmosphere of 92.5% air/7.5% CO_2 at 37 °C. The number of HUVEC at confluency was 2×10^5/well. Confluent HUVEC were washed three times in serum-free RPMI 1640, and were incubated in 0.5 ml of the same medium with or without the stimuli and/or paclitaxel at 37 °C in 7.5% CO_2.

MN cells were obtained from whole blood collected from healthy donors by Lymphoprep sedimentation as previously described.[23] MN cell preparations were resuspended in RPMI 1640 medium supplemented with penicillin (100 U/mL) and streptomycin (100 μg/mL) at cell concentration of 3×10^6 cells/mL in the presence of the different stimuli with or without paclitaxel in sterile, pyrogen-free stoppered test tubes at 37 °C. The monocytes in this population were 25–30%, as assessed by non-specific esterase staining.

In all instances, the concentrations of paclitaxel used were not associated with cytotoxic effects as determined by LDH release experiments.

2.3. Procoagulant activity assay

After incubation, cells were disrupted by three freeze-thaw cycles before testing for procoagulant activity by a one-stage clotting time test as previously described.[23] The

procoagulant activity was characterised using the monoclonal anti-TF antibody HTF1. The inhibition of the activity observed with the anti-TF antibody indicates this activity to be attributable to TF.

Results were expressed in arbitrary units (U) by comparing with a standard curve obtained using a human brain thromboplastin standard kindly donated by Dr. L. Poller, Manchester, UK. This preparation was assigned a value of 1000 U for a clotting time of 20 s.

2.4. TF antigen

To determine TF antigen, the cells were harvested and lysed in TBS, pH 8.5 containing 1% Triton x-100 overnight. Cell debris was pelleted by centrifugation at 100,000*g* for 60 min at 4 °C. The protein content of the supernatant was determined by BCA protein assay kit (PIERCE) and adjusted to 2 mg/ml. Samples were then used for ELISA, according to the manufacturers' instructions.

2.5. Quantitative Real-time reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA was isolated from cells with the RNeasy Plus Mini Kit (Qiagen). The RNA was reverse transcribed with random primers with the high capacity cDNA Reverse Transcription Kit (Applied Biosystems). The real-time RT-PCR was performed in 96-well plates on the ABI Prism 7500 Sequence Detection System (Applied Biosystems) with TaqMan Universal PCR Master Mix and Assays-On-Demand (Applied Biosystems), with a final reaction of 20 μl. The PCR primers and 6-carboxyfluorescein (FAM) probe for the F3 gene were purchased as Assays-On-Demand. The assay number for the target gene was Hs00175225_m1. Human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as an endogenous control (Applied Biosystems). All the samples were performed in triplicate. The relative expression of the target genes was normalised to the level of GAPDH in the same cDNA by the use of the 2-DDCT method.[24]

2.6. Statistical analysis

The results are given as mean values ± SEM. Differences between two groups were tested for significance using Student's *t* test for paired observations. ANOVA followed by Dunnett's or Tukey's test when appropriated was used for multiple comparisons.

3. Results

3.1. Effect of paclitaxel on TF expression in MDA-MB-231 cells

MDA-MB-231 cells constitutively expressed both TF activity and antigen, which were not modified upon exposure to LPS (data not shown). When the cells were incubated with paclitaxel, a dose-dependent inhibition of TF activity was observed (Fig. 1). At the highest paclitaxel concentration (2 nM), a moderate but statistically significant decrease in TF antigen was detected by ELISA (Fig. 1, inset).

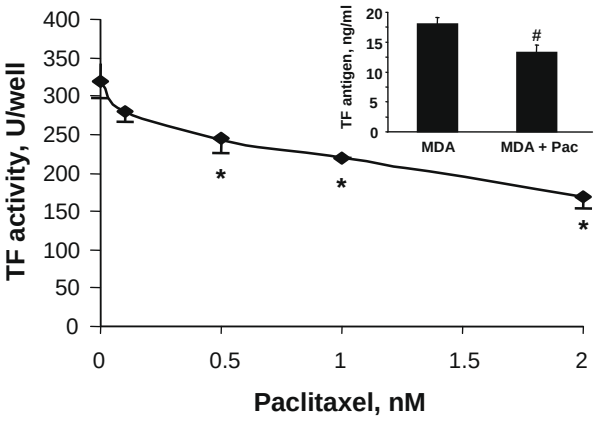

Fig. 1 – Effect of paclitaxel on MDA-MB 231 cells TF activity and antigen. MDA-MB 231 cells were incubated with the depicted concentrations of paclitaxel for 6 h at 37 °C. At the end of incubation, cells were disrupted by freezing and thawing, and TF activity was measured by a one-stage clotting time. Inset: cells were incubated with 2 nM paclitaxel (Pac) for 6 h, lysed as described in Section 2 and tested for TF antigen levels by ELISA. Data are mean ± SEM of 4 experiments. Global effect of paclitaxel on tissue factor (TF) activity, $p = 0.0001$ (ANOVA). *Significant ($p < 0.05$) contrast following Dunnet's test (reference level: untreated cells). #$p < 0.05$ for TF antigen of paclitaxel-treated cells compared to untreated cells.

3.2. Effect of paclitaxel on MN cells TF activity and antigen

Procoagulant activity was barely detectable in freshly isolated MN cells, and exposure to paclitaxel (2 nM) did not affect the activity levels (0.012 ± 0.003 and 0.016 ± 0.005 U/3 × 10^5 MN, $n = 4$, respectively). When MN cells were incubated with 0.1 μg/ml LPS for 6 h at 37 °C, a strong TF activity could be observed (Fig. 2A).

When paclitaxel was present during the incubation, TF activity was reduced in a concentration-dependent way, reaching a plateau at 1–2 nM with approximately a 60% activity inhibition (Fig. 2A). Preincubation of the drug for 1 h prior to LPS treatment gave similar results (not shown). The inhibition of TF activity by paclitaxel was accompanied by a significant decrease in TF antigen as assessed by ELISA (Fig. 2A, inset).

3.3. Effect of paclitaxel on HUVEC TF activity and antigen

A very low amount of TF activity was detectable in HUVEC under basal conditions, and no modification could be induced by paclitaxel (0.16 ± 0.04 versus 0.16 ± 0.04 U/well, $n = 4$, respectively). Conversely, exposure of HUVEC to 1 μg/ml LPS for 6 h at 37 °C induced substantial TF activity (Fig. 2B). Similar to what was observed with MN cells, when paclitaxel was present during the incubation, TF activity was reduced in a concentration-dependent way, reaching a plateau between 1 and 2 nM (Fig. 2B). As for MN cells, paclitaxel preincubation did not modify the rate of inhibition (not shown). Once again, the inhibition of TF activity by paclitaxel was accompanied by a significant decrease in TF antigen (Fig. 2B, inset).

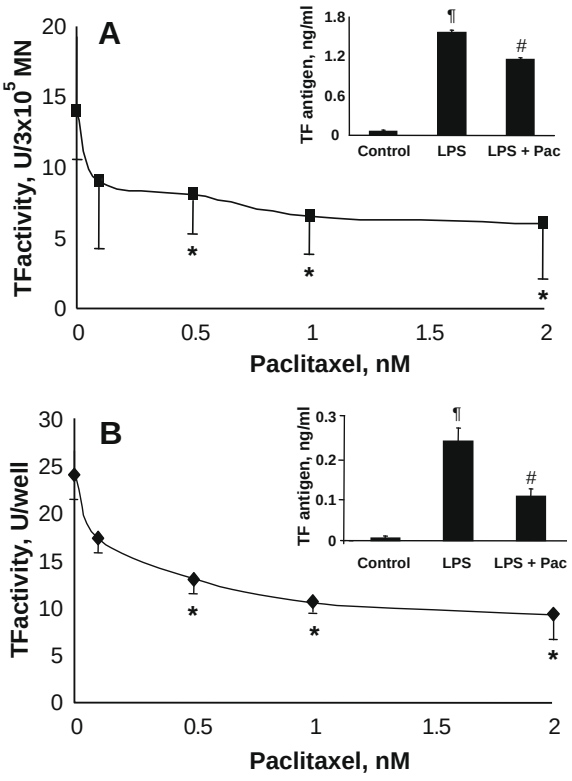

Fig. 2 – Effect of paclitaxel on TF activity and antigen in LPS-stimulated MN cells and HUVEC. MN cells (A) and HUVEC (B) were incubated with or without LPS (0.1 μg/ml and 1 μg/ml, respectively), in the presence of different concentrations of paclitaxel for 6 h at 37 °C. At the end of incubation samples were treated for the measurement of TF activity. Inset: cells were incubated without (control) or with LPS (0.1 μg/ml for MN cells and 1 μg/ml for HUVEC) in the presence or in the absence of 2 nM paclitaxel (Pac) for 6 h, lysed and tested for TF antigen levels by ELISA. Data are mean ± SEM of 5 experiments. Global effect of paclitaxel on TF activity, $p = 0.001$ and $p = 0.0001$ (ANOVA) for MN cells and human umbilical vein endothelial cells (HUVEC), respectively. *Significant ($p < 0.05$) contrast following Dunnet's test (reference level: untreated cells). Insets: global effect of paclitaxel on TF antigen, $p = 0.0001$ (ANOVA) for both MN cells and HUVEC. #Significant ($p < 0.05$) contrast for TF antigen of paclitaxel-treated compared to LPS-treated cells and ¶LPS-stimulated cells compared to control.

3.4. *Paclitaxel modulation of TF mRNA levels in MDA-MB-231 cells, MN cells and HUVEC*

To examine the effect of paclitaxel on TF mRNA levels, MDA-MB-231 cells were incubated with or without paclitaxel, while MN cells and HUVEC were exposed to LPS in the presence or in the absence of paclitaxel. After 1 h, the cells were washed, mRNA was extracted and studied by real-time RT-PCR.

A constitutive expression of TF mRNA could be observed in MDA-MB 231 cells. The presence of paclitaxel partially prevented TF mRNA expression (Fig. 3A).

TF mRNA expression was not detectable in resting MN cells and HUVEC. In contrast, exposure of MN cells to LPS resulted in a strong TF mRNA induction (288.27 ± 48.5-fold relative to the respective amount in unstimulated cells, $n = 4$, $p < 0.05$). A similar behaviour could be observed when HUVEC were incubated without or with LPS (90.3 ± 2.74-fold, $n = 4$, $p < 0.001$).

When paclitaxel, which had no effect if incubated alone with the cells, was present together with LPS, a decrease in TF mRNA could be observed in both MN cells and HUVEC (Fig. 3B and C).

3.5. *Effect of paclitaxel on TF activity of TNF-α- and IL-1β-stimulated MN cells and HUVEC*

To investigate whether paclitaxel could modulate TF activity in the presence of agonists different from LPS, MN cells and

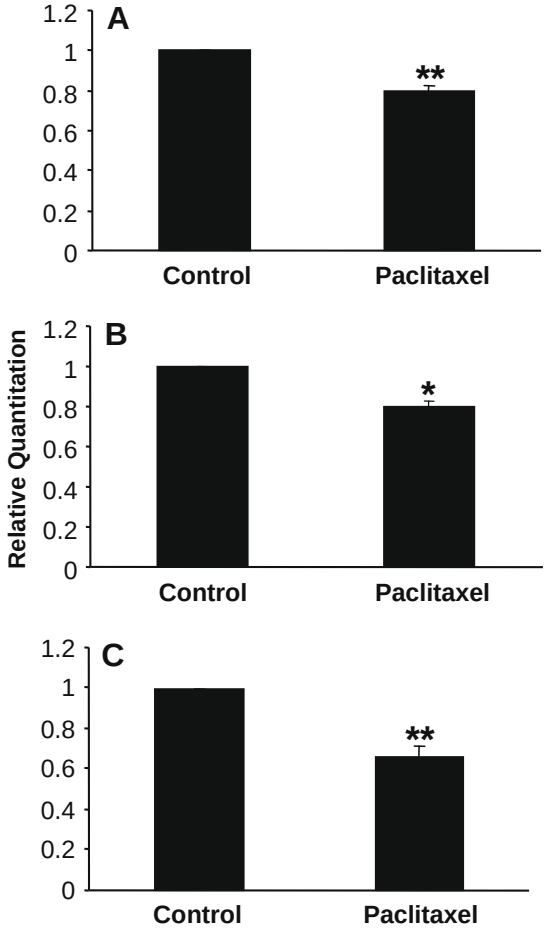

Fig. 3 – Effect of paclitaxel on TF mRNA levels in MDA-MB-231 cells, and LPS- stimulated MN cells and HUVEC. MDA-MB-231 cells, and LPS-stimulated MN cells and HUVEC (0.1 μg/ml, and 1 μg/ml, respectively) were exposed to 2 nM paclitaxel (Pac) for 1 h at 37 °C. At the end of incubation, total RNA was extracted from cells, and mRNA levels were evaluated by real-time RT-PCR as described in Section 2. mRNA levels are expressed as normalised ratio, assigning a value of 1 to the control samples. A) MDA-MB-231 cells (control: resting cells); B) MN cells (control: LPS-stimulated MN cells) and C) HUVEC (control: LPS-stimulated HUVEC). The columns represent the mean ± SEM of 4 different experiments. *$p < 0.01$; **$p < 0.005$.

HUVEC were incubated for 6 h at 37 °C with or without TNF-α and IL-1β in the presence of paclitaxel. Exposure of MN cells (Fig. 4A) and HUVEC (Fig. 4B) to either recombinant TNF-α or IL-1 β resulted in an induction of TF activity. Similar to what was observed when LPS was used, the presence of paclitaxel reduced TF activity in both the cell types.

3.6. *Effect of paclitaxel on IL-6 and IL-1β expression in MDA-MB-231 cells, MN cells and HUVEC*

To test whether the effect of paclitaxel was specific for TF, the possible modulation of the expression of the proinflammatory cytokine IL-6 was investigated. As can be seen in Fig. 5A, exposure of MDA-MB-231 cells to paclitaxel did not affect the constitutive release of IL-6. Similarly, paclitaxel could neither induce IL-6 expression from resting cells nor modulate its LPS-induced expression (Fig. 5B and C). Analogous results were obtained when IL-1β secretion was examined (Fig. 6). Results obtained with HUVEC are not shown, since IL-1β levels were below the detection limit of the assay in all instances.

4. Discussion

Thrombosis is a frequent complication of malignancy, and is the second most important cause of death in cancer patients.[25,26] Activation of blood clotting in cancer patients plays a role in cancer growth, particularly in metastatic development. Specific factors which have been implicated in the clotting activation associated with malignancy consist mainly in tumour or host cell procoagulants. Malignant cells constitutively express tissue factor.[10–12] Tumour cells themselves can induce the expression of TF by host cells such as endothelial cells or monocyte/macrophages.[18–22] Thus, TF on tumour or host cell surface represents a major mediator of clotting activation at the tumour–host interface.

In this report, we show for the first time that paclitaxel downregulates the constitutive expression of TF in the highly metastatic human breast carcinoma cell line MDA-MB-231. TF expression downregulation is exerted at the level of activity, antigen and mRNA.

Although under normal conditions monocytes and endothelial cells do not express TF antigen or activity, these cells, upon appropriate stimulation, can be induced to synthesise and express TF on their membranes.[27] In our work, we demonstrate that paclitaxel inhibits MN cells and HUVEC TF expression induced by LPS and the proinflammatory cytokines IL-1β and TNF-α. Similar to what was observed with MDA-MB-231 cells, TF inhibition in these cells is exerted at the level of TF activity, antigen and mRNA.

Among the chemotherapeutic compounds used in clinical oncology, paclitaxel, the anti-tumour agent isolated from the bark of Pacific yew trees, has achieved a strong standing for the beneficial outcomes determined in patients with tumours,

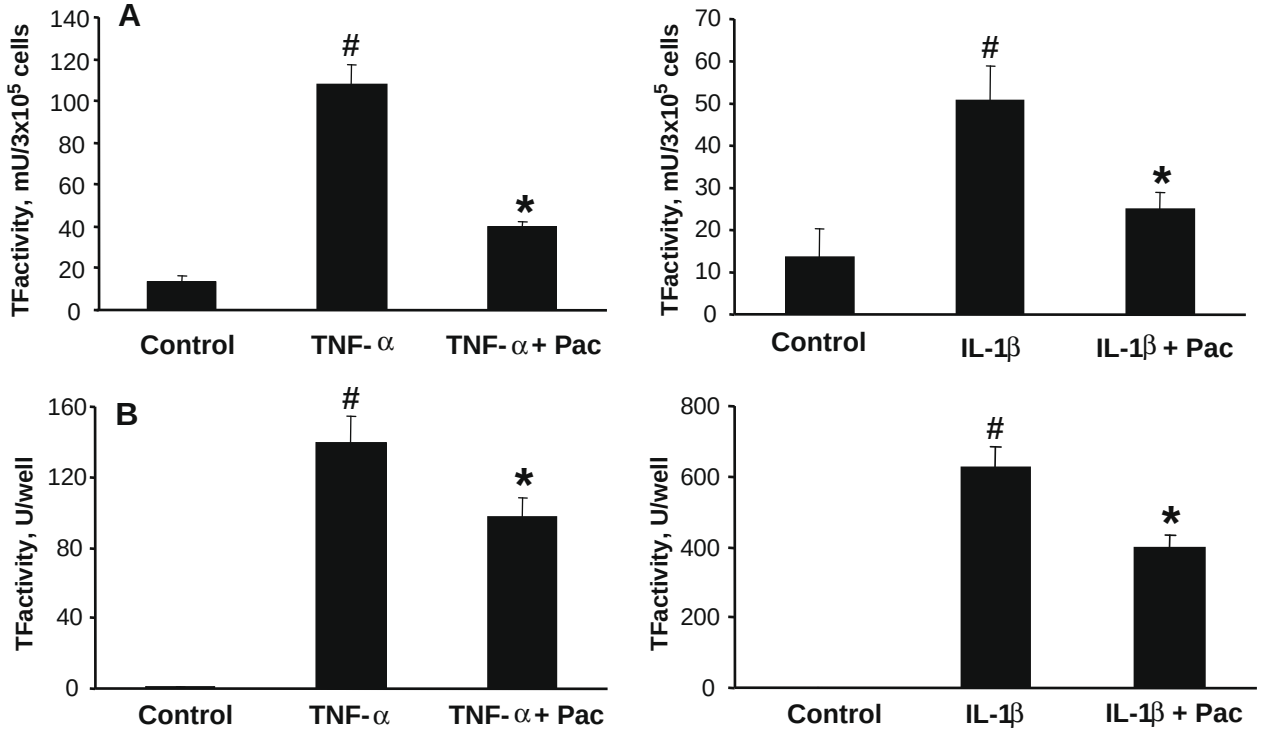

Fig. 4 – Effect of paclitaxel on TF activity of TNF-α and IL-1β-stimulated MN cells and HUVEC. MN cells (A) and HUVEC (B) were incubated with or without TNF-α (40 and 10 ng/ml, respectively) or IL-1β (40 and 10 ng/ml, respectively) and with or without paclitaxel (Pac) 2 nM for 6 h at 37 °C. At the end of incubation, cells were disrupted by freezing and thawing, and TF activity was measured by a one-stage clotting time. Data are mean ± SEM of 5 experiments. Global effect on TF activity, $p = 0.0001$ (ANOVA) in all instances. *Significant ($p < 0.05$) contrast following Tukey's test for TF activity of paclitaxel-treated compared to TNF-α- and IL-1β-stimulated cells and #TNF-α- and IL-1β-stimulated cells compared to control.

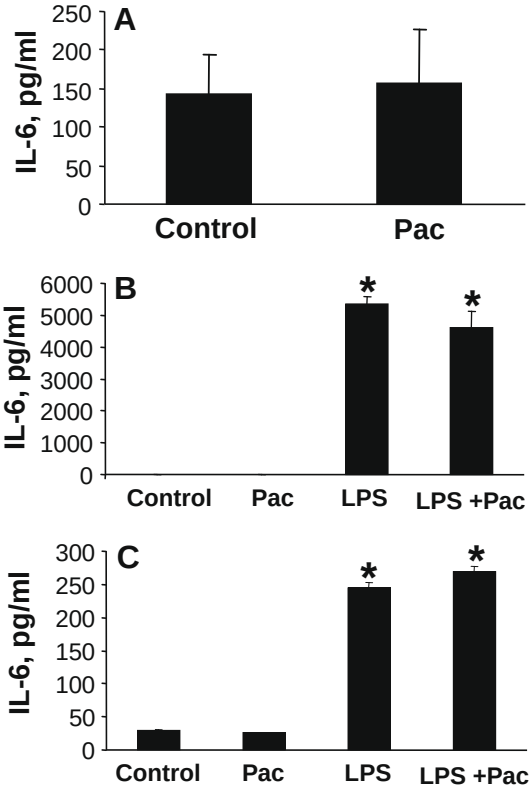

Fig. 5 – Effect of paclitaxel on IL-6 levels in MDA-MB 231 cells, MN cells and HUVEC. MDA-MB-231 cells (A), and MN cells (B) and HUVEC (C) resting or LPS-stimulated (0.1 and 1 μg/ml, respectively) were exposed to 2 nM paclitaxel (Pac) for 6 h at 37 °C. At the end of incubation, the conditioned medium was collected, centrifuged to eliminate cell debris and IL-6 antigen was evaluated by ELISA. Data are mean ± SEM of 4 experiments. Global effect on IL-6 levels, $p = 0.0001$ (ANOVA) for both MN cells and HUVEC. *Significant ($p < 0.05$) contrast following Tukey's test for IL-6 levels of LPS– and LPS + paclitaxel-treated MN cells and HUVEC compared to controls or cells exposed to paclitaxel alone.

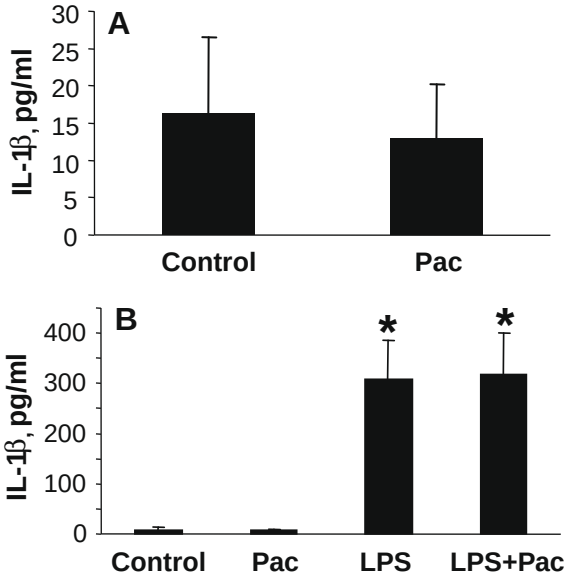

Fig. 6 – Effect of paclitaxel on IL-1β levels in MDA-MB 231 cells and MN cells. MDA-MB-231 cells (A), and MN (B) resting or LPS-stimulated cells (0.1 μg/ml) were exposed to 2 nM paclitaxel (PAC) for 6 h at 37 °C. At the end of incubation, the conditioned medium was collected, centrifuged to eliminate cell debris and IL-1β antigen was evaluated by ELISA. Data are mean ± SEM of 3 experiments. Global effect on IL-1β levels, $p = 0.005$ (ANOVA) for MN cells. *Significant ($p < 0.05$) contrast following Tukey's test for IL-1β levels of LPS– and LPS + paclitaxel-treated MN cells compared to controls or cells exposed to paclitaxel alone.

such as ovarian, breast and lung cancer, refractory to other methods of treatment.[1,28] The proposed mechanism of action of this diterpenoid resides in its binding to the β subunit of tubulin heterodimers, the consequent stabilisation of microtubules and cell cycle arrest.[2,29] Paclitaxel, in addition to the impairment of mitosis, exhibits pleiotropic effects on different cells. Notably, several lines of evidence have suggested that paclitaxel may exert an antiangiogenic effect. In an *in vivo* mouse model paclitaxel inhibited the angiogenic response induced by tumour cell supernatant, while in an *in vitro* mouse model it downregulated endothelial cell motility, invasiveness and cord formation at concentrations which did not affect endothelial cell proliferation.[30] Moreover, in an *in vivo* mouse model paclitaxel-incorporated photocrosslinked chitosan hydrogels effectively inhibited both tumour growth and angiogenesis.[31] In this respect, it has been shown that paclitaxel could modulate the expression of vascular endothelial growth factor (VEGF), which is the most potent regulator of angiogenesis. Paclitaxel downregulated the expression of VEGF in the murine breast cancer cell line Met-1 transplanted in nude mice or maintained in cell culture.[32] In human adenocarcinoma cell lines, the expression of the VEGF gene induced by radiation was suppressed by paclitaxel pre-treatment.[33] Moreover, paclitaxel downregulated VEGF in solid tumours from patients who were affected by invasive ductal carcinoma.[34] Interestingly, it has been reported that paclitaxel at ultra low concentrations (0.1–100 pM) inhibits angiogenesis without affecting cellular microtubule assembly,[8] since paclitaxel requires a concentration range of approximately 10–30 nM to polymerise tubulin.[28]

These reports, together with the evidence that TF is closely linked to the angiogenic process, with mechanisms that are either dependent or independent of blood clotting activation, confer plausibility to the effect of paclitaxel in modulating TF expression. Indeed, in addition to an essential role for TF in embryonic vasculature angiogenesis,[35] a strong interrelationship between TF and VEGF in pathological angiogenesis has been widely documented (reviewed in Refs. [36,37]). It should be mentioned that in vascular cells around human breast cancer cells the *in situ* detection of TF correlated with the malignant phenotype, suggesting TF as a marker for the initiation of angiogenesis in cancer.[21] In our experimental conditions, inhibition of TF was achieved at paclitaxel concentrations as low as 0.5 nm. Although we did not study microtubule structure, it is tempting to speculate that downregulation of TF expression may be independent of tubulin polymerisation.

In addition to its contribution to the angiogenic process, paclitaxel can induce the expression of genes encoding enzymes that are involved in cell proliferation, apoptosis and inflammation.[28] In particular, paclitaxel has been reported to induce the expression of the proinflammatory cytokines IL-1β[3,4,38] and TNF-α[3,38,39] in monocytes/macrophages and in some cancer cell lines. In our experimental conditions, no upregulation of IL-6 and IL-1β could be detected in MDA-MB-231 cells, in agreement with a previous report in which MDA-MB-231 challenge with paclitaxel did not induce IL-1β expression.[4] Lack of induction was also observed in host cells, namely MN cells and HUVEC. This is not surprising, considering that gene induction by paclitaxel requires concentrations of 10–30 μM[28] a range that is greater by 10,000-fold than that used in our study.

In addition, paclitaxel could not modulate IL-6 and IL-1β in LPS-stimulated MN cells and HUVEC, indicating that the effect of paclitaxel on TF was specific.

At odds with our results, a previous report showed that paclitaxel enhanced the activated endothelial TF expression.[40] Different experimental conditions could help in understanding the apparent discrepancy with our results; indeed, these authors used human aortic endothelial cells, which have been shown to behave differently from their venous counterpart concerning the mechanisms underlying site-specific thrombosis.[41,42] Moreover, thrombin, which was used as an agonist, could induce signalling pathways different from those elicited by LPS or by the inflammatory cytokines we made use of. Although the authors could detect the increase in TF at paclitaxel doses higher than those utilised in our study, it should be mentioned that in our experimental model a plateau was reached at approximately 2 nM in all instances, and an increase in concentration up to 10 nM did not alter the response. In addition, a very recent paper demonstrated an induction of TF expression in arterial smooth muscle cells in response to paclitaxel.[43] Once again, the different cell target and the higher paclitaxel concentrations used do not allow direct comparison with our results.

In conclusion, our results demonstrate that paclitaxel inhibits TF activity/expression by cancer and host cells, supporting the hypothesis that the anti-tumour effect of this chemotherapeutic agent may, in part, be mediated by the capacity of this drug to modulate the procoagulant potential of cancer and host cells. Moreover, the inhibition of endothelial cell TF could open interesting perspectives for the antiangiogenic activity of paclitaxel.

Knowledge presently available on the multiple effects of TF pathway on tumour cell responses should allow to better define the role of TF among the potential target of antimetastatic therapy.

Conflict of interest

None declared.

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