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Biochimica et Biophysica Acta 1763 (2006) 1125–1134
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
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The C-terminal CD47/IAP-binding domain of thrombospondin-1 prevents camptothecin- and doxorubicin-induced apoptosis in human thyroid carcinoma cells

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Received 22 June 2006; received in revised form 28 July 2006; accepted 1 August 2006
Available online 5 August 2006

Abstract

Camptothecin and doxorubicin belong to a family of anticancer drugs that exert cytotoxic effects by triggering apoptosis in various cell types. However there have only been few investigations showing that matricellular proteins like thrombospondin-1 (TSP-1) could be involved in the underlying mechanism of this cytotoxicity. In this report, using Hoechst reagent staining, reactive oxygen species production and caspase-3 activity measurement, we determined that both camptothecin and doxorubicin induced apoptosis in human thyroid carcinoma cells (FTC-133). On the one hand, we demonstrated that camptothecin and doxorubicin inhibited TSP-1 expression mainly occurring at the transcriptional level. On the other hand, drug-induced apoptosis determined by western blot analysis for PARP cleavage and caspase-3 activity measurement, was significantly decreased in presence of exogenous TSP-1. In order to identify the sequence responsible for this effect, we used the CD47/IAP-binding peptide 4N1 (RFYVVMWK), derived from the C-terminal domain of TSP-1, and known to play a role in apoptosis**.** Thus, in presence of 4N1, camptothecin and doxorubicin-induced pro-apoptotic activity was considerably inhibited. These findings suggest that induction of apoptosis by camptothecin or doxorubicin in FTC-133 cells is greatly dependent by a down-regulation of TSP-1 expression and shed new light on a possible role for TSP-1 in drug resistance.

Keywords: 4N1; Camptothecin; Doxorubicin; Apoptosis; Thyroid carcinoma cell

1. Introduction

The thyroid gland is necessary for the development and homeostasis of vertebrates [1]. However, numerous thyroid pathologies exist including benign thyroiditis and thyroid carcinoma which represents the most common endocrine malignancy, accounting for the majority of deaths from endocrine cancers. After the Chernobyl accident in April 1986, an important number of thyroid cancers was diagnosed particularly in children. The incidence rate has been multiplied by 10 between 1990 and 2000 depending on the exposure area and an important number of new cases over the next 50 years are expected [2]. Conventional therapy consists of surgical resection, radioiodine ^{131}I therapy, TSH-suppressive thyroxin treatment and additional chemotherapy in the case carcinoma became insensitive to iodine therapy [3,4]. Among the cytotoxic compounds pointed out as potent therapeutics against cancer, we used camptothecin and doxorubicin. Camptothecin acts as a DNA topoisomerase I inhibitor that blocks DNA synthesis [5]. Doxorubicin is an anthracycline having multiple potential mechanisms of action including inhibition of DNA topoisomerase I and II, inhibition of helicases, generation of toxic free radicals, alteration of membrane structure and function and

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doi:10.1016/j.bbamcr.2006.08.001

endonucleolytic cleavage activity [6–8]. Both drugs have been described to induce apoptosis in different cell types [9–13]. Apoptosis plays an important role during development and normal turn-over of adult tissues and a decrease in the rate of apoptosis might have severe pathological consequences that facilitate tumor growth [14]. Thus, apoptosis appears to confer little obvious therapeutic benefit and several anticancer drugs exert their cytotoxic effect by this way [15].

Some of our previous studies evidenced that modification in the expression of the matricellular multidomain glycoprotein thrombospondin-1 (TSP-1) could play a critical role in the regulation of thyroid cell proliferation, migration and invasion [16]. The role of this protein in cancer and apoptosis is both complex and controversial [17], conflicting functions are described, TSP-1 being either pro- or anti-apoptotic according to cell type [18,19]. Moreover, a peptide derived from the C-terminal domain of TSP-1 (4N1) has been proposed to play a role in apoptosis [20]. This VVM motif-containing peptide mediates its effects through the binding to integrin-associated protein CD47 [21].

In the present work, we studied the apoptotic effects of doxorubicin and camptothecin in a poorly differentiated follicular thyroid carcinoma cell line FTC-133, showed the effects of these drugs on TSP-1 expression and secretion and evidenced a potential anti-apoptotic role for TSP-1 in this cell line. Moreover, the C-terminal globular domain of TSP-1 that binds to integrin-associated protein (IAP or CD47) was shown to play an important role in the observed protective effect of the protein.

2. Materials and methods

2.1. Materials

Doxorubicin was obtained from Farmitalia (Milano, Italy). TSP-1 was from Calbiochem. 4N1 (RFYVVMWK) was purchased from BACHEM (Weil am Rhein, Germany), 4NGG peptide (RFYGGMWK) was synthesized by Dr. Patigny IFR 53 and anti-CD47 antibody B6H12 from BD Biosciences (Le Pont de Claix, France). Dulbecco's modified Eagle's medium/F-12 and trypsin were purchased from Invitrogen (Cergy Pontoise, France). Bovine foetal serum was from Dutscher (Brumath, France). PARP (#9542) antibodies were from Cell Signaling (Beverly, USA). Monoclonal TSP-1 Ab-11 antibodies (#MS-1066) were from Labvision. β-actin antibodies (A5441) were from Sigma. ECL western blotting detection reagents was from Amersham (Freibourg, Germany). All other products, including camptothecin, were from Sigma (St. Louis, MO, USA).

2.2. Cell line and culture conditions

FTC-133 is a human follicular thyroid carcinoma derived cell line (ECACC 94060901) obtained from a lymph node metastasis of a single patient [22]. This cell line expresses most proteins present in differentiated thyroid tissue such as thyroglobulin (Tg), thyrotropin receptor (TSHr), thyroid transcription factors TTF-1 or Pax-8 [23,24]. However, it does not express TPO and hNIS like many thyroid cancers [24]. Moreover FTC-133 exhibit a p53 mutation which seems to confer cells the ability to grow in culture [25]. Cells were routinely grown in Dulbecco's modified Eagle's medium/F-12 (1:1) supplemented with 10% heat-inactivated foetal calf serum, 100 μg/mL streptomycin and 100 IU/mL penicillin. Cells were cultured in 75 cm^2 flasks at 37 °C in a 5% CO_2–95% air–water saturated atmosphere. Trypsinization was performed at confluence. Isolated cells were then cultured in 96-well culture plates for cell viability and luciferase assay and in 6-well culture plates for western blot analysis, mRNA extraction, caspase assay or Hoechst staining. Experiments were performed during the exponential phase of cell growth and each control is a DMSO control.

2.3. Cell viability determination

Cells were plated in microwell plates at 10^4 cells/mL and incubated until they reached the logarithmic growth phase. The spent medium was aspirated from cells and replaced with serum free medium. After 24 h incubation, camptothecin or doxorubicin was added. At indicated times, 10% (v/v) UptiBlue (Uptima, Interchim, Montluçon, France) was added. Plates were incubated for an additional 3 h and reduction was then measured spectrofluometrically (λex: 530–560 nm; λem: 590 nm). Results were calculated as percent of controls as follows: (Experimental absorbance / untreated control absorbance) × 100.

2.4. Hoechst reagent staining

Cell monolayers cultured as described above were treated with 5 μM camptothecin or doxorubicin for 12 h. Cells were then stained with propidium iodide (10 μg/mL) and Hoechst 33258 reagent (20 μg/mL) for 15 min at room temperature and photographed under fluorescence microscopy (λex: 351.1–363.8 nm; λem: 390–480 nm). Apoptotic cells, which contained condensed chromatin fragments, were scored in three replicate wells for each treatment and each assay was repeated at least three times. Results are expressed as percentage of the total cell number.

2.5. Spectrofluorimetric assay of ROS production

Cells treated for 0 to 6 h in the presence of 5 μM camptothecin or doxorubicin and untreated control cells, were incubated with 2 μM of dihydroethidium (λex: 360 nm; λem: 420 nm) for 10 min, washed with phosphate-buffered saline, and then analysed by spectrofluorometry in RPMI-1640 medium without phenol red. In the presence of ROS, dihydroethidium is oxidized to ethidium and fluoresces in red (λem: 640 nm). The 640/420 nm fluorescence intensity ratio permits to measure the production of ROS in living cells.

2.6. Caspase-3 activity

After incubation, cell pellets were washed twice with PBS and scrapped with ice-cold lysis buffer as provided by Promega (caspACE assay kit) and caspase-3 activity was measured accordingly: 50 μg of cytosolic fraction were incubated with caspase-3 colorimetric substrate that absorbs at 405 nm following its cleavage. Absorbance was measured with a multichannel plate reader (Metertech. inc. Σ960).

2.7. Western blotting

Cultured cells were made quiescent by serum starvation for at least 24 h before treatment. Conditioned media were collected, centrifuged at 3000×*g* for 5 min and total protein was measured using BCA assay from Uptima (Interchim, Montluçon, France). Cells were then washed with ice-cold PBS, lysed in ice cold buffer containing 10 mM Tris pH 7.4, 150 mM NaCl, 5 mM EDTA, 1 mM Na_3VO_4, 1 mM dithiothreitol, 10 μg/mL leupeptin, 10 μg/mL aprotinin, 10% glycerol, 1% Brij (v/v), placed on ice for 20 min and then centrifuged at 14,000×*g* for 15 min at 4 °C. Equal amounts of proteins (conditioned media or whole cell extracts) were resolved by 10% SDS-PAGE gels and transferred to nitrocellulose membranes and probed with the appropriate antibodies (monoclonal anti-TSP-1: dilution 1/400, polyclonal anti-PARP: dilution 1/1000, or anti-β-actin antibodies: dilution 1/8000). Horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG were used as secondary antibodies (dilution 1/4000 and 1/10,000 respectively) and proteins were detected using an enhanced chemiluminescence (ECL) kit.

2.8. Transfection and luciferase assay

FTC-133 cells (5×10^6) grown in 96 well microplates (PerkinElmer), were transiently transfected with the pGL3-TSP-1 promoter sequence − 1290/+ 750

cloned upstream of the luciferase (LUC) gene using Lipofectamine 2000 reagent following the protocol provided by the supplier (Life Technologies, Rockville, MD, USA). One hour following transfection, cells were treated or not with camptothecin or doxorubicin at the concentrations and times as indicated. At the end of the incubation, cells were washed with PBS, lysed with 100 µl FireLite dual reporter lysis buffer (PerkinElmer Life Sciences, France) and luciferase activities were measured with the PerkinElmer TopCount microplate counter.

2.9. Reverse transcription-polymerase chain reaction

RT-PCR was performed on total RNA prepared by RNeasy® Mini kit (QIAGEN). 1 µg of total RNA was reverse transcribed using AMV reverse transcriptase and oligo(dT)$_{15}$ primer from Promega. Amplification was performed using PCR Master Mix and *Taq* DNA polymerase from Promega. The optimal reaction conditions were: 30 cycles, 56 °C for TSP-1 and 25 cycles, 50 °C for S26. Specific primer pairs were, for TSP-1: forward 5′-CTC AGG AAC AAA GGC TGC TC-3′ reverse: 5′-ACT CCT GAA TGT GGC AGG TC-3′; for S26: forward 5′-GTG CGT GCC CAA GGA TAA GG-3′, reverse 5′-ATG GGC TTT GGT GGA GGT CG-3′.

2.10. Statistics

Data are mean ± S.D. of three independent experiments (Student's *t* test), p levels of <0.05 were considered significant.

3. Results

3.1. Camptothecin and doxorubicin affected FTC-133 cell viability in a time-dependent manner

The potential cytotoxic effects of camptothecin and doxorubicin on FTC-133 thyroid carcinoma cells were assessed using the UptiBlue viable cell counting assay, as described in Materials and methods. The cells were treated with various doses of each drug for different times (3, 6, 12 or 24 h). As indicated in Fig. 1, both camptothecin and doxorubicin exhibited cytotoxic effects in a time-dependent manner when used at a final concentration of 5 µM. Although lower doses from 0.5 µM also decreased cell viability (unpublished data), we decided to use each drug at 5 µM for the following investigations to reach nearly 50% cell death after 12 h stimulation.

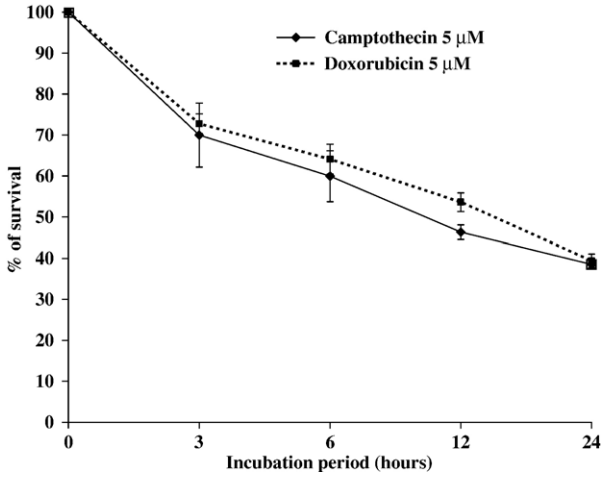

Fig. 1. Effect of camptothecin and doxorubicin on cell viability. FTC-133 cells were incubated with either 5 µM camptothecin or 5 µM doxorubicin for the indicated times. Cell viability was determined by Upti-blue assay as described in Materials and methods. Results are expressed as percent of control and represent means ± standard deviations (S.D.) of at least three independent experiments.

3.2. Camptothecin and doxorubicin induced thyroid carcinoma cell apoptosis

FTC-133 cells were incubated with or without camptothecin or doxorubicin for 12 h. Cells were double stained with propidium iodide and Hoechst reagent and examined by microscopy (Fig. 2A). When exposed to both drugs, FTC-133 cells appeared highly susceptible to apoptosis. Thyroid carcinoma cells indeed exhibited the distinctive morphology of apoptotic cells including nuclear shrinkage and desegregation (42 ± 2% in drug treated cells versus 0% in control cells). To confirm that these observed morphological changes were caused by apoptosis induction, similarly treated-cells were used to quantify the reactive oxygen species (ROS) production, known to play a main role in apoptosis induction, [26] (Fig. 2B) and the caspase-3 activity (Fig. 2C). The capacity of both drugs to induce an oxidative stress was investigated using the fluorescent probe dihydroethidium. Indeed, Fig. 2B showed that both drugs were able to induce ROS production as soon as 4 h, reaching nearly 140% after 6 h of treatment. Moreover, as shown in Fig. 2C, both camptothecin and doxorubicin increased the cleavage of caspase-3 substrate up to four-fold after 8 h incubation, reporting an acute caspase-3 activity.

3.3. Effect of camptothecin and doxorubicin on TSP-1 expression and secretion

Quiescent FTC-133 cells were transiently transfected with the pGL3-basic vector containing the − 1290/+ 750 sequence of the human TSP-1 promoter inserted upstream of the *firefly* luciferase gene [27,28]. These thyroid carcinoma cells were treated with or without camptothecin or doxorubicin for different times (Fig. 3A). The results showed a significant decrease in the luciferase activity from 3 h for doxorubicin (15% inhibition) and from 6 h for camptothecin (15% inhibition). At 12 h, the remaining luciferase activity was only 20% in camptothecin-treated cells and 5% in doxorubicin-treated cells as compared to control. The decrease in TSP-1 promoter gene activity induced by both drugs was correlated with a reduction in TSP-1 mRNA expression and protein secretion (Figs. 3B and C). After 8 h of treatment, TSP-1 mRNA expression was reduced with camptothecin and completely inhibited in the presence of doxorubicin (Fig. 3B). In addition, using Western blot analysis, we measured a two-fold reduction in the secretion of TSP-1 protein after 12 h incubation with camptothecin or doxorubicin and no more secreted TSP-1 was detected from 24 h while β-actin kept a steady state level (Fig. 3C). These results led us to investigate whether TSP-1 could play a pivotal role in the apoptotic program triggered by camptothecin and doxorubicin. To address this question, we used purified human TSP-1 for further investigations.

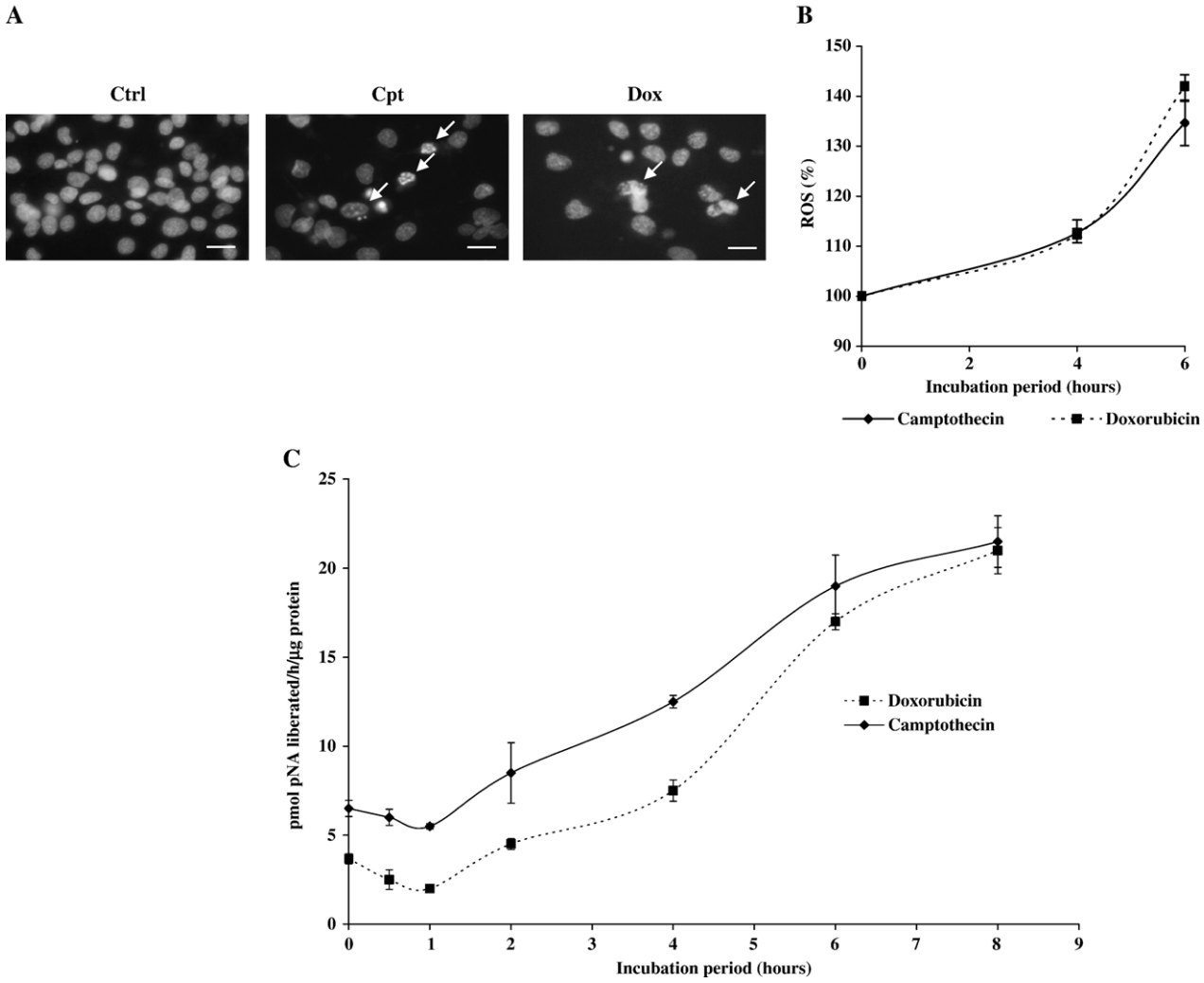

Fig. 2. Evaluation of the effect of camptothecin and doxorubicin on apoptosis induction. (A) Morphologic examination after Hoechst and propidium iodide staining of FTC-133 cells cultured for 12 h alone (Ctrl) or in the presence of either 5 μM camptothecin (Cpt) or 5 μM doxorubicin (Dox). Original magnification ×200. Scale bars, 50 μm. White arrows indicate apoptotic bodies. (B) ROS production in drug-treated cells. FTC-133 cells were treated for the indicated periods with 5 μM camptothecin or 5 μM doxorubicin before incubation with 2 μM dihydroethidium for 10 min. Data were normalized with respect to control values and results are expressed as percentage of initial ROS amount set at 100%. Results are given as means±S.D. of three independent experiments. (C) Effect of camptothecin and doxorubicin on caspase-3 activity. FTC-133 cells were treated for the indicated times with 5 μM camptothecin or 5 μM doxorubicin. Caspase-3 activity was determined as described in Materials and methods. Results are expressed in pmol pNA (para-nitroaniline) liberated per hour and per μg protein and are shown as means±S.D. of three independent experiments.

3.4. Effect of TSP-1 on camptothecin or doxorubicin-induced carcinoma cell apoptosis

PARP, is known to facilitate cellular disassembly, remains one of the main cleavage targets of caspase-3 in vivo and is considered as a good marker for cells undergoing apoptosis. As shown in Fig. 4A, treatments with camptothecin or doxorubicin for 8 h induced PARP cleavage. An upregulation of PARP expression was observed mainly under doxorubicin treatments, as frequently reported when using such toposiomerase [8]. When the cells were pre-treated 30 min with TSP-1 before drug addition, PARP cleavage was not any more detected indicating that TSP-1 was capable of preventing the drug-induced PARP processing. Moreover, the caspase-3 activity was assessed in similar conditions (Fig. 4B). The results showed that TSP-1 protected carcinoma cells from camptothecin or doxorubicin-induced caspase-3 activation.

3.5. Effect of 4N1 peptide on camptothecin or doxorubicin-induced apoptosis

It has been demonstrated that the biological effect of TSP-1 was connected to its binding to cell surface receptors such as CD47 or CD36 [29,30]. In FTC-133 cells, a high level of CD47 receptor expression compared to a low level of CD36 was detected (data not shown). Further experiments were also carried out using the CD47 agonist peptide RFYVVMWK (4N1), derived from the C-terminal domain of TSP-1, and the scramble peptide 4NGG (RFYGGMWK) that served as control peptide [21]. Furthermore, to determine whether the CD47

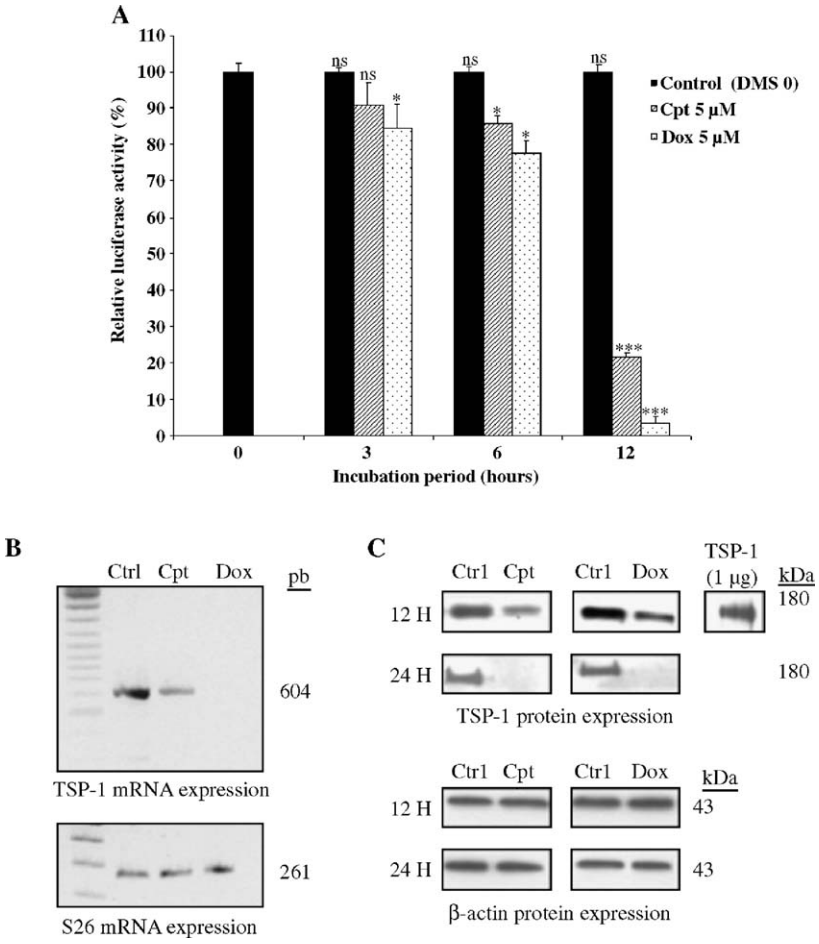

Fig. 3. Effect of camptothecin and doxorubicin on TSP-1 expression and secretion. (A) Quiescent FTC-133 cells transiently transfected with the − 1290/+ 750 bp TSP-1 promoter construct were treated for the indicated times without or with 5 µM camptothecin or 5 µM doxorubicin. Luciferase activities are expressed in relative units to drug stimulation. Basal luciferase activity (DMSO stimulated sample) was established as 100%. The data are shown as the means ± standard deviations (S.D.) of at least three independent experiments. SD bars marked with asterisks differ significantly (Student's *t* test) from control (*$p<0.05$, ***$p<0.001$, ns, non-significant). (B) FTC-133 cells treated with camptothecin or doxorubicin for 8 h were used for TSP-1 mRNA expression. The constitutively expressed S26 "housekeeping" gene was used as a normalizing control. (C) Cells treated for 12 and 24 h were used to study TSP-1 protein secreted in the culture medium by Western blot. β-actin antibody was used as a control.

receptor could be involved in the TSP-1-dependent regulation of cell apoptosis, the blocking anti-CD47 antibody B6H12 [31] was used. Effects of the peptides and B6H12 antibody on FTC-133 cell growth were assessed by using UptiBlue viable cell counting assay, as described in Materials and methods. Cells were treated with both drugs in addition of 4NGG, 4N1, or 4N1 and B6H12 for various periods (3, 6, 12 or 24 h). The results presented in Fig. 5 showed that 4NGG scramble peptide exhibited no effect on camptothecin or doxorubicin-induced growth arrest. On the contrary, 4N1 treatment protected cells from camptothecin and doxorubicin-induced growth arrest. In the presence of B6H12 antibody, the 4N1 peptide became unable to prevent the drug-dependent inhibition of cell growth, thus demonstrating the molecular role of CD47 in the observed 4N1 protective effect. Moreover, western blot analysis (Fig. 6A) showed that in the presence of the 4N1 peptide, the cleaved PARP fragment was not any more detected in FTC-133 cells treated with camptothecin or doxorubicin. In the presence of B6H12, the 4N1 peptide did not prevent the camptothecin or the doxorubicin-induced PARP cleavage. These results were confirmed by measuring caspase-3 activity under the same conditions (Figs. 6B and C). Indeed, in presence of the 4N1 peptide, the camptothecin-induced caspase-3 activity was abolished and the treatment with 4N1 alone (without drug) exhibited no effect on caspase-3. The same effect in a lesser extend was observed when 4N1 was used in combination with doxorubicin, thus indicating that this treatment remained significant in preventing doxorubicin-induced caspases-3 activity. As previously observed for cell growth and PARP processing, blocking the CD47 membrane receptor avoided the protective effect of 4N1 on drug-stimulated caspase-3 activity. Identical results were obtained by quantifying the 17 kDa cleaved caspase-3 pro-apoptotic form by immunoblotting (data not shown).

4. Discussion

The role of thrombospondin in the process of programmed cell death has recently came into focus and attracted much attention from scientists interested in altering the behavior of

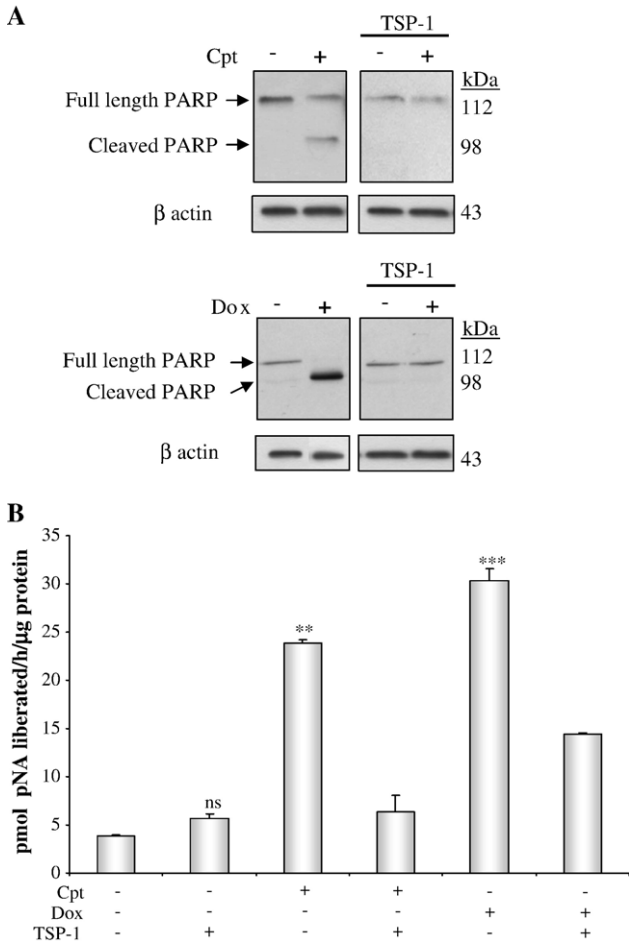

Fig. 4. Effect of TSP-1 on camptothecin and doxorubicin induced apoptosis. Both PARP cleavage (A) and caspase-3 activity (B) were analysed. Cells were treated for 8 h in the absence or presence of 5 μM camptothecin, 5 μM doxorubicin or 2 μM TSP-1 alone or combined. PARP cleavage was determined by Western blot analysis and caspase-3 activity was measured as described in Materials and methods. Results are expressed in pmol pNA liberated per hour and per μg protein and are shown as means±S.D. of three independent experiments. S.D. bars marked with asterisks differ significantly from control (**$p<0.01$, ***$p<0.001$, ns, non-significant). S.D. bars marked with circles differ significantly from the corresponding values without TSP-1 (°°$p<0.01$).

human cancer cells. In the present study, we established that TSP-1-derived peptide 4N1 exhibited anti-apoptotic properties against thyroid tumoral cells and decreased sensitivity to apoptosis induced by camptothecin and doxorubicin.

First, we demonstrated that treatment of human thyroid carcinoma cells with camptothecin or doxorubicin led to elevated cytotoxic events associated with reactive oxygen species generation and correlated to an increased caspase-3 activity and induced PARP processing. This is in agreement with other data supporting that the mechanism of cell death mediated by camptothecin or doxorubicin were related to apoptosis in a range of human metastases diseases such as T-cell lymphoma, colorectal, breast, ovarian, renal or prostate cancer cells [32–34]. However, in the context of thyroid diseases, camptothecin has only been investigated until today in the treatment of medullary thyroid carcinoma (MTC) representing only 5% of all thyroid malignancies, but never in metastatic thyroid follicular carcinoma (FTC) [35]. Doxorubicin is recognized as the most effective cytotoxic drug against anaplasic thyroid carcinoma (ATC), one of the most aggressive human malignancies, and therefore this drug is extensively used to cure thyroid cancer [36,37]. However, to our knowledge, only two investigations were really interested to determine whether doxorubicin treatments cause cell apotosis in follicular thyroid carcinoma (FTC) and the corresponding results remained highly controversial [38,39]. Indeed, although Yeung and collaborators [38] failed to detect a doxorubicin-induced cell death in FTC cells, Massart and colleagues [39] developed the concept of a doxorubicin-induced Fas-mediated apoptosis corroborating our results.

Second, we demonstrated for the first time that camptothecin or doxorubicin-induced apoptosis was correlated with TSP-1 down regulation, mainly occurring at the transcriptional level. Similarly, a recent investigation conducted in our laboratory underlined that overexpression of the PTEN tumor-suppressor gene leading to induction of thyroid carcinoma apoptosis was also correlated with a significant down-regulation of TSP-1 expression at both RNA and protein levels [16]. These results

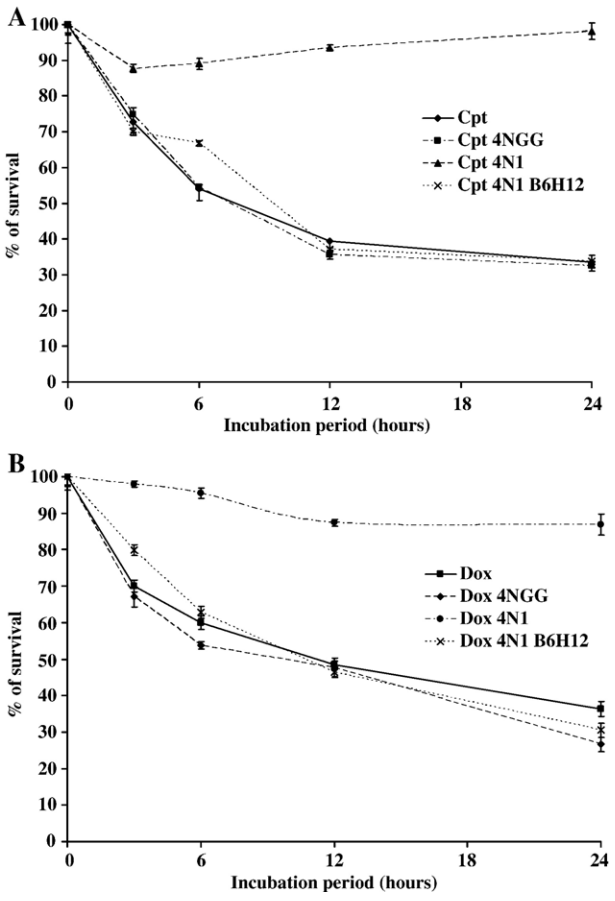

Fig. 5. Effect of 4N1 on camptothecin- and doxorubicin-induced growth inhibition. FTC-133 cells were incubated with either camptothecin or doxorubicin at 5 μM along with 100 μM 4N1 or 4NGG and B6H12 antibody at 100 μg/ml for the indicated times. Cell viability was determined by Upti-blue assay as described in Materials and methods. Results are expressed as percent of control and represent means±standard deviations (S.D.) of at least three independent experiments.

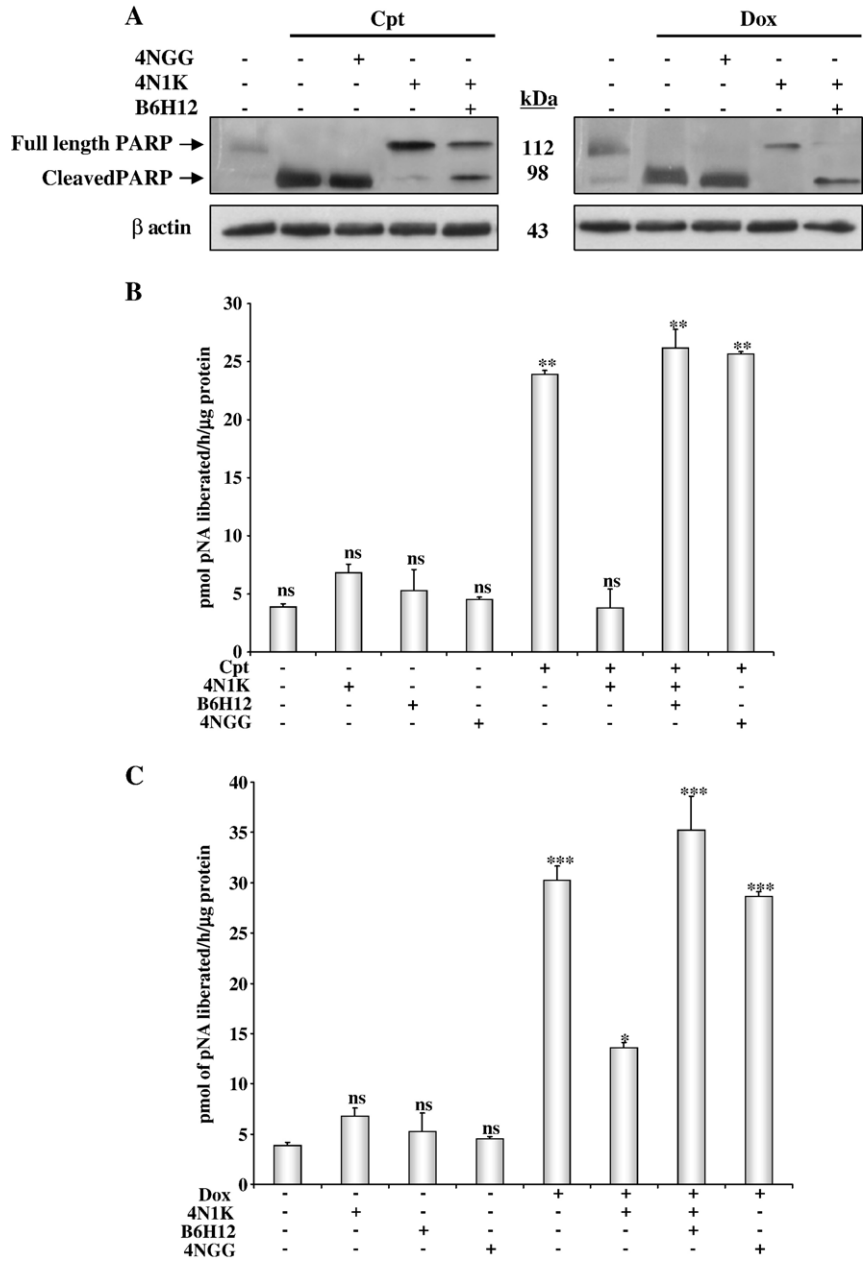

Fig. 6. Effect of 4N1 peptide on camptothecin or doxorubicin induced apoptosis. Cells were treated for 8 h. Camptothecin or doxorubicin were present as indicated at 5 μM along with 100 μM 4N1 or 4NGG and B6H12 antibody at 100 μg/ml. Western blot analysis was performed for PARP cleavage (A) and Caspase-3 activity was measured as described in Materials and methods (B and C). Results are expressed in pmol pNA substrate liberated per hour and per μg protein and are shown as means±S.D. of three independent experiments. SD bars marked with asterisks differ significantly from control (*$p<0.05$, **$p<0.01$, ***$p<0.001$, ns, non-significant).

led us to investigate a possible anti-apoptotic role for TSP-1 in FTC cells. For the first time our results shed new light on the anti-apoptotic properties of both the mature TSP-1 protein and the synthetic TSP-1-derived peptide 4N1 in thyroid cancer. We provided evidences that both treatments were capable of inhibiting cell death triggered by camptothecin and doxorubicin by interfering with the caspase-3 pathway. Consistent with recent observations [40], we identified the CD47 receptor as potential membrane protein relaying 4N1-mediated control of tumor cell death; CD36 being undetectable under our experimental conditions (data not shown). In opposite to our findings, the main recent laboratory analyses reported a critical function for TSP-1 in the stimulation of cell apoptosis in several cancerous cells and tissues [41–44]. For example, recent data supplied proofs that TSP-1 exerted direct pro-apoptotic effects on leukaemia cells by means of a caspase-dependent mechanism mediated by CD36 [45] or by a caspase-independent way depending to CD47 binding [45,46]. Additionally, although we observed that TSP-1 dramatically decreased the efficiency of camptothecin-induced apoptosis in thyroid carcinoma, others proposed that TSP-1 could be used in combination with campthotecin analogues to increase inhibition of tumor growth

in human colon cancer [47]. In the same way, studies performed with synthetic peptides derived from TSP-1 designated them as strong inducers of tumor cell death but focused mostly on two heparin-binding peptides located within the NH2-terminal and type 1 repeats [42,48]. In this study, we were mostly interested in the biological activity of the TSP-1-derived C-terminal 4N1 peptide. In the light of these results, the TSP-1-dependent regulation of cancer cell death must be cautiously apprehended. Indeed, it seems to be directly related to the nature of the synthetic TSP-1-derived peptide and to the differential distribution of the specific membrane receptors that directly depends on the cellular context.

Cancer cell resistance to chemotherapy is a major concern in clinical oncology, resulting in reduced cancer cell apoptosis, increased tumor growth and decreased patient survival. Thyroid cancers are considered as highly chemoresistant malignancies what engaged most of the non-surgical therapeutic efforts on radioiodine therapy [49]. Here, we developed the concept that the levels of TSP-1 could directly drive the thyroid cancer resistance to doxorubicin treatment, thus impairing the efficiency of such chemotherapy in clinical practice [39,50]. This is in agreement with recent data showing that FTC238 thyroid cells, exhibiting a higher endogenous TSP-1 level than FTC133, were the least sensitive to doxorubicin treatment [39]. Consistent with our results, TSP1 was previously linked to disease recurrence and decreased patient survival [17,51]. For example, TSP-1 was recently considered as a poor prognostic factor for survival in colorectal metastases [52] and in invasive bladder cancer [53]. Some reports investigated TSP-1 expression level in clinical thyroid cancer cases. One study showed no significant difference in mean TSP-1 mRNA expression in in vivo thyroid cancers in comparison to normal specimens [54]. However, another study demonstrated that TSP-1 expression was reduced in correlation with the increasing aggressiveness of different thyroid lesions [55]. Another report evaluating 75 papillary thyroid cancers, demonstrated that TSP-1 expression was inversely correlated with invasiveness [56]. Moreover, this contradictory theory was evoked in other cell system and several approaches that were designed to increase the levels of thrombospondin are being developed for the treatment of cancer [57]. Indeed, patients with TSP-1-negative tumors appeared to present a poor prognosis in colon cancer [58] and overexpression of TSP-1 in mice lacking endogenous TSP-1 was reported to suppress tumor growth [59]. Additionally, 4N1-containing proteins in tumor tissues were considered as a predictive marker for patient survival with renal carcinoma since 4N1-positive tumors were locally confined and of remarkably smaller size [60]. Recent studies described that some cancers may develop the ability to counterbalance their own secretion of pro- or anti-tumoral factors [61–63] which could explain the apparent conflicting results. Some tumor cells were shown to bypass the expected inhibitory effect of TSP-1. For exemple, human breast cancer cells could override the antiangiogenic effects of TSP-1 in vivo by increasing vascular endothelial growth factor (VEGF) expression [64]. Scarpino et al. [65] demonstrated that TSP-1 expression was under the control of hepatocyte growth factor (HGF) in papillary thyroid carcinomas and in malignant gliomas overexpression of TSP-1 and TGFβ increase the biologic malignancy [66]. In FTC-133 cells, a continuous inhibition of TSP-1 under drug treatment was observed and addition of TSP-1 or its derived peptide 4N1 in camptothecin- or doxorubicin-treated cells exhibited an unexpected anti-apoptotic effect. Whether other growth factors are implicated remains to be demonstrated. Altogether, according to the cellular environment specificity, TSP-1 could be either significantly associated with a worse prognosis or considered as an interesting marker for survival in human metastases.

Finally, our results provide new information that may explain one component of the drug-resistance phenotype in thyroid tumoral diseases and support the idea that TSP-1 could be helpful for predicting recurrence and survival outcome in patients affected by such pathologies. Understanding how biological factors such as TSP-1 are capable of driving chemotherapy resistance and decreasing patient survival only in some cancers will be of great interest during the next years to potentially enhance therapeutic response and to identify the best clinical protocol to be used for each patient, improving efficacy and tolerance.

Acknowledgements

This work was supported by MENRT, CNRS and by grants from the “Ligue de la Marne” and the “Region Champagne-Ardenne”.

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