PERGAMON

European Journal of Cancer 36 (2000) 1043–1049

European Journal of Cancer

www.ejconline.com

Opposite effects of antimicrotubule agents on c-*myc* oncogene expression depending on the cell lines used

V. Bourgarel-Rey [a,1], S. El Khyari [b,1], O. Rimet [a], B. Bordas [a], N. Guigal [a], D. Braguer [a], E. Seree [a], Y. Barra [a,*], C. Briand [a]

[a] *UPRES-A CNRS 6032 Faculté de Pharmacie, 27 Bd Jean Moulin, 13005 Marseille, France*
[b] *Laboratoire de Microbiologie et Biotechnologie, Université Chouaib Doukkali, El Jadida, Maroc*

Received 2 August 1999; received in revised form 4 January 2000; accepted 5 January 2000

Abstract

We investigated the expression of c-*myc* in HT29-D4, HBL100 and Caco-2 cells treated with microtubule stabilising (paclitaxel) or depolymerising agents (vinblastine, nocodazole). After induction by epidermal growth factor (EGF), c-*myc* expression decreased in HT29-D4 cells treated with all the antimicrotubule agents. In HBL100 and Caco-2, when microtubules were stabilised with paclitaxel, c-*myc* expression also decreased. In contrast, its expression increased after treatment with depolymerising agents. In both cell lines, we also observed that depolymerising agents alone induced c-*myc* expression whilst paclitaxel had no effect. This mRNA induction was confirmed at the protein level. In HT29-D4, no variation of c-*myc* expression was observed. Then, we showed that the increase of mRNA level was due to activation of gene transcription. These results indicate that modulation of c-*myc* expression varied depending on the cell lines used and the type of antimicrotubule agents. This work provides a potential link between the microtubular network and c-*myc* gene expression.

Keywords: Antimicrotubule agents; c-*myc*; Transcription activation

1. Introduction

Some antitumour drugs act on the microtubular network and are termed antimicrotubule agents. Paclitaxel (Taxol™:TAX) and docetaxel stabilise microtubules and promote their assembly whilst vinca-alkaloids and colchicinoids inhibit tubulin polymerisation. These drug actions result in the blockage of cells in the G2/M phase. At low concentrations, all antimicrotubule agents inhibit microtubule dynamics [1,2]. Moreover, other studies reported that paclitaxel may have an additional effect on the expression of some genes (tumour necrosis factor α [3] or interleukin-1 gene [4]). Taxoids, vinca-alkaloids and colchicine induce apoptosis following mitotic block but the apoptotic pathway has not yet been determined [5,6]. Paclitaxel may mediate apoptosis via induction of p34cdc2 kinase or phosphorylation of Bcl-2 protein [7]. It has been shown that these agents increase the level of transcriptionally active p53 in some cell lines [8].

The c-*myc* proto-oncogene, usually implicated in cell transformation, differentiation and cell cycle progression also plays a central role in some forms of apoptosis [9]. c-myc, with its partner max, functions as a transcription factor actuating apoptosis when low amounts of survival factors are present [10]. Like other members of the nuclear proto-oncogene family (c-*fos*, c-*jun*...) c-*myc* is transiently activated after serum stimulation of quiescent cells [11]. Most human tumours exhibit genetic alterations in myc family members that result in the deregulation of myc expression [12]. This oncogene is regulated by transcriptional and post-transcriptional mechanisms [13]. An interaction between c-myc protein and tubulin was described in HL60 [14]. We confirmed this observation in HT29-D4 cells (human colon carcinoma cell line) and we also showed that pretreatment with antimicrotubule agents decreased c-*myc* induction by fetal bovine serum (FBS) [15]. However, we feel that this observation should be extended to other cell lines

* Corresponding author. Tel.: +33-4-91-83-55-94; fax: +33-4-91-80-26-12.
E-mail address: yves.barra@pharmacie.univ-mrs.fr (Y. Barra).
[1] V. Bourgarel-Rey and S. El Khyari contributed equally to this work.

0959-8049/00/$ - see front matter
PII: S0959-8049(00)00042-3

and we have attempted to investigate the molecular mechanism involved. We chose HBL100 and Caco-2 cells that were respectively of either different or the same tissue origin as HT29-D4.

2. Materials and methods

2.1. Cell culture

Human colon adenocarcinoma HT29-D4 cells and HBL100 cells, normal human epithelial mammary cells, were cultured in Dulbecco's modified essential medium (DMEM) supplemented with 10% FBS, penicillin/streptomycin, L-glutamine and sodium pyruvate. The human colon adenocarcinoma Caco-2 cells were cultured in DMEM supplemented with 15% FBS, penicillin/streptomycin, L-glutamine, sodium pyruvate and non-essential amino acids. The doubling time of each cell line was approximately 20–24 h. Cells (at 80% of confluence) were starved for 24 h with FBS-free medium and then treated with various drugs for different periods of time depending on the cell line.

2.2. Reagents

Stock solutions of paclitaxel (TAX) (Sigma, St Quentin, France) and nocodazole (NOC) (Sigma) were prepared at a 10^{-2} M concentration in dimethyl sulphoxide (DMSO), vinblastine (VLB) (10^{-3} M) and EGF (10 mg/ml; Biowhittaker, Emerainville, France) were prepared in sterile distilled water and kept frozen until use.

2.3. Evaluation of c-myc expression

2.3.1. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

Total cellular RNA was extracted by the Chomczynski and Sacchi method [16], with the RNAXEL kit (Eurobio, Les Ulis, France). One microgram of total RNA was used for reverse transcription with random primers. We then co-amplified c-*myc* and β_2 microglobulin (β_2m), the internal standard, with Taq polymerase (Eurobio). The primer sequences were:

c-*myc* (Sense): 5′ TACCCTCTCAACGACAGCAG-CTCGCCCAACTCCT 3′

c-*myc* (Antisense): 5′ TCTTGACATTCTCCTCGG-TGTCCGAGGACCT 3′

β_2m (Sense): 5′ CCGACATTGAAGTTGACTTAC 3′

β_2m (Antisense): 5′ ATCTTCAAACCTCCATGATG 3′.

PCR was carried out in a Perkin Elmer system 2400. The reaction conditions included a denaturation at 93°C for 2 min, followed by 24 cycles of denaturation at 92°C, 10 s, annealing at 52°C, 30 s and extension at 72°C, 45 s with increments of 20 s each cycle and one final cycle of extension at 72°C for 7 min. We determined that with 24 cycles, the amplification was in the linear range. The amplified products were separated by electrophoresis on a 2% agarose gel. The DNA bands were visualised by ethidium bromide staining, and the image was digitalised. c-*myc* expression was normalised to β_2m transcript. This was noted as the REL (Relative Expression Level) which equals the densitometric value of c-*myc*/densitometric value of β_2m. The percentages of variation of c-*myc* expression reported in the text were the mean values of five experiments.

2.3.2. Western blot

Cells were lysed in a buffer containing 2.5% sodium dodecyl sulphate (SDS), 5% β-mercaptoethanol, 10% glycerol, 62.5 mM Tris pH 6.8 and protease inhibitors [17]. Proteins (20 μg) were separated by electrophoresis on a 10% SDS–polyacrylamide gel and transferred to nitrocellulose membranes. Membranes were incubated with the anti-c-myc mouse antibody (9E10; Zymed, Montrouge, France) at 1/1000 dilution, exposed to anti-mouse peroxidase-labelled secondary antibody and visualised with Western blot chemoluminescence reagent (ECL Amersham, Orsay, France). The amount of protein loaded on the gel was controlled after staining the membrane with ponceau S red.

2.3.3. Flow cytometry analysis

Cells were trypsinised and fixed in cold methanol (70%, 20 min at −20°C), washed and treated with phosphate buffered saline (PBS) solution containing 60 μg/ml RNAse (37°C, 30 min). The cells were then stained with propidium iodide (20 μg/ml) for 30 min at room temperature. DNA content was measured by flow cytometry (Becton Dickinson FAC Sort) and the percentage of cells in the G0/G1, S and G2/M cell-cycle phases was calculated [18].

2.3.4. Immunofluorescence

Cells were treated with different drugs during 7 h, then fixed in 3% paraformaldehyde, permeabilised with 70% methyl alcohol and acetone at −20°C. After blocking with PBS–bovine serum albumin (BSA) (1%), cells were incubated with mouse anti-c-myc monoclonal antibody (9E10; Zymed) diluted at 1/100. The secondary antibody used was a fluorescein-conjugated goat anti-mouse antibody (Amersham; dilution 1/20).

2.4. Activation of c-myc transcription

2.4.1. CAT assays

pMpCAT [19] containing c-*myc* promoter sequences from nucleotides −2328 to +936 was obtained from D. Levens (NIH, Bethesda, MD, USA). Cells in 6-well plates were transiently transfected in the log phase using lipofectin (Life Technologies, Cergy Pontoise, France).

They were incubated with 1 μg pMpCAT DNA for 18 h. Cells were stimulated with the drugs 24 h later and harvested after 48 h treatment. CAT expression was then evaluated by the amount of CAT protein using the CAT ELISA system (Boehringer, Meylan, France).

The transfection efficiency between dishes was verified by transfecting cells with pCMV/βgal. β-galactosidase activity was evaluated by spectrofluorimetry as previously described [20].

As a control, we transfected cells with $pBLCAT_2$ containing the *CAT* gene under the control of the tyrosine kinase promoter. With this plasmid, we did not observe any CAT expression variation upon our treatment conditions (data not shown).

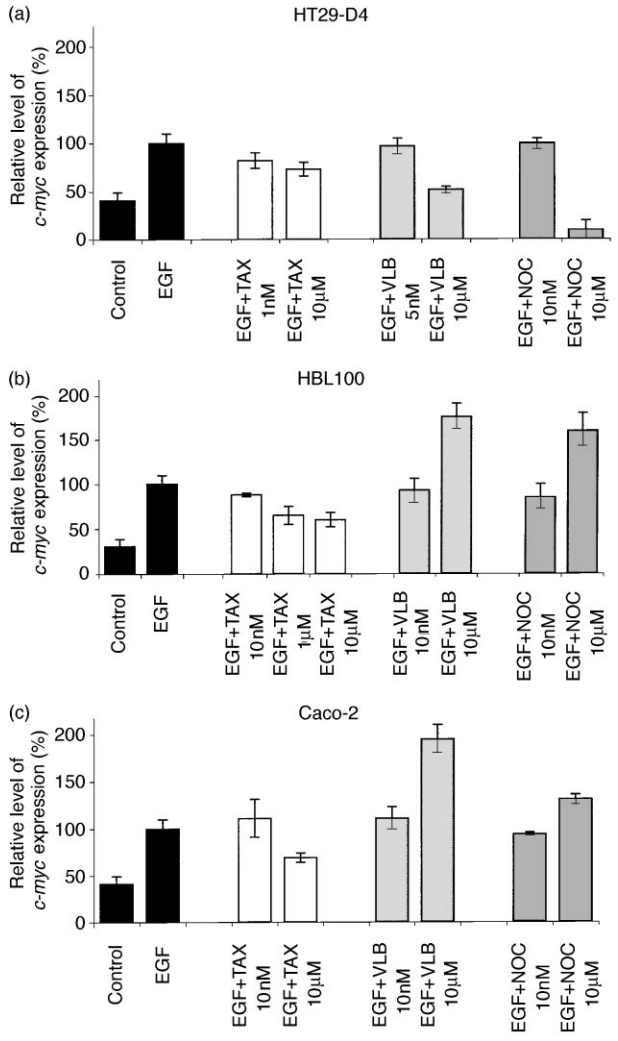

Fig. 1. Effects of antimicrotubule agents on c-*myc* induction by EGF. (a) HT29-D4 cells: After 24 h of serum starvation, cells were pretreated for 4 h with the drugs. At $t=0$ h EGF (20 ng/ml) was added in the presence of TAX, VLB, NOC and c-*myc* expression was evaluated 2.5 h later by RT-PCR. (b) HBL100 cells: Cells were pretreated with TAX, VLB and NOC at different concentrations 4 h before EGF addition, c-*myc* expression was then evaluated 1.5 h later. (c) Caco-2 cells: Cells were pretreated with TAX, VLB and NOC 4 h before EGF addition, c-*myc* expression was then evaluated 3 h later.

2.4.2. Evaluation of hnRNA level

A RT-PCR assay was applied to amplify a region of the c-*myc* heterogeneous nuclear RNA (hnRNA) transcript. RT-PCR on hnRNA was used to detect transcriptional activation as a substitute for the nuclear run-on assay, hnRNA being unaffected by mRNA stabilisation or destabilisation [21]. Total RNA was prepared using the High Pure RNA extraction kit (Boehringer) which includes a DNAse treatment to degrade the genomic DNA. RT was performed as described above. For the PCR reaction the primers were chosen in two different intronic sequences.

HnRNA *myc* Sense: 3′AGA CCC CTT TAA CTC AAG ACT GCC TC 5′

HnRNA *myc* Antisense: 3′AAA ATG GGA AAG GTA TCC AGC CGC C 5′.

Absence of amplification products with non-reverse transcribed RNA was used as a control for potential contamination by genomic DNA. We then amplified c-*myc* hnRNA and $\beta_2 m$. The reaction conditions included a denaturation at 93°C for 4 min, followed by 20 cycles of denaturation at 93°C, 30 s, annealing at 62°C, 30 s and extension at 72°C, 30 s followed by 16 cycles of 93°C, 30 s, annealing at 62°C, 30 s and extension at 72°C, 30 s with increments of 20 s each cycle. The level of c-*myc* hnRNA was assessed and normalised to $\beta_2 m$ transcript as previously described. We verified the specificity of the amplified products using several restriction enzymes. The control experiment allowing distinction of transcriptional events and altered processing rate consisted of treating the cells with α-amanitin (2 μg/ml), a transcription inhibitor. The percentages of variation of c-*myc* hnRNA expression reported in the text were the mean values of three experiments.

3. Results

3.1. Effects of antimicrotubule agents on c-myc induction by EGF

Fig. 1a shows the effects of antimicrotubule agents on c-*myc* mRNA level in HT29-D4 cells that were serum-starved for 24 h and 2.5 h after the addition of EGF. At this time, the maximal c-*myc* expression was observed in this cell line after the addition of growth factors [15]. c-*myc* Expression was increased approximately 3-fold compared with basal levels. No significant effect was observed with low-doses of drugs (10 nM TAX, 5 nM VLB and 10 nM NOC). Then, we tested high-doses that modify the microtubular network. With 1 μM TAX we observed a slight decrease (approximately 28%) in c-*myc* induction by EGF. VLB at 10 μM (the dose at which the paracrystals are formed) and NOC at 10 μM decreased c-*myc* expression by 50% and 90%, respectively.

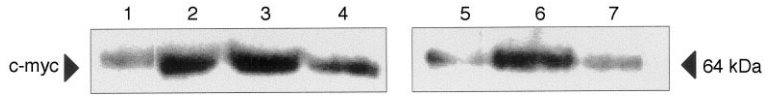

Fig. 2. Immunoblotting analysis of the effects of the antimicrotubule agents on c-myc protein level with (lanes 1–4) or without (lanes 5–7) EGF addition in HBL 100 cells. Lanes 1 and 5: 24 h after serum starvation; lane 2: EGF induction for 1.5 h; lane 3: 4 h pretreatment with 10 μM VLB, then EGF addition in the presence of VLB for 1.5 h; lane 4: 4 h pretreatment with 10 μM TAX, then EGF addition in the presence of TAX for 1.5 h; lane 6: 10 μM VLB-treated cells for 5.5 h; lane 7: 10 μM TAX-treated cells for 5.5 h.

We also investigated the effect of drugs in HBL100 cells (Fig. 1b). We first established in these cells the kinetics of EGF induction of c-*myc* expression and found that the maximum value was reached at an earlier time (1.5 h) (data not shown) than it was in HT29-D4 cells. The low-doses of drugs (10 nM TAX, 10 nM VLB and 10 nM NOC) had no significant effect on c-*myc* induction. Treatment of HBL100 cells with high-doses of TAX (1 and 10 μM) inhibited c-*myc* induction by approximately 40% (Fig. 1b). Conversely, high-doses of the depolymerising agents in these cells increased c-*myc* expression: 80% with 10 μM VLB and 60% with 10 μM NOC.

Since HT29-D4 and HBL100 cell lines behave differently in response to high-doses of depolymerising agent, we tested a third cell line, Caco-2. In these cells, maximal c-*myc* induction was reached 3 h after EGF addition (data not shown). We found that at this time 10 μM TAX decreased c-*myc* induction by 30% whilst 10 μM VLB and 10 μM NOC increased it by 95 and 30%, respectively (Fig. 1c).

With regard to the flow cytometry analysis (data not shown), it appeared that for the three cell lines and all agents tested, the low-doses of drugs used did not block cells in the G2/M phase after 24 h treatment. These doses were 10 nM except for VLB in HT29-D4 cells. Since in these cells, 10 nM VLB blocked in G2/M phase, we used a lower dose (5 nM). The high-doses used (1 or 10 μM) for all compounds blocked cells in G2/M phase after 24 h treatment.

The result presented in Fig. 2 shows in HBL100 cells that, compared with the control (lane 1) EGF increased c-myc protein level 3-fold (lane 2). As we found for mRNA, pretreatment with 10 μM VLB (lane 3) increased the c-myc protein level by 30% and a pretreatment with 10 μM TAX (lane 4) decreased it (40%).

3.2. *Effects of antimicrotubule agents alone on c-*myc *basal expression and localisation*

Fig. 3 showed that in HBL100 cells, 10 μM VLB (lane 2) and 10 μM NOC (lane 3) increased c-*myc* expression approximately 4.2±1.0-fold and 3.2±1-fold, respectively, and that no effect was detectable with 10 μM TAX (lane 4). In Caco-2 cells, 10 μM VLB (lane 2) and 10 μM NOC (lane 3) increased c-*myc* expression 3.8±1.1-fold and 2±0.4-fold respectively. In these cells, as in HBL100 10 μM TAX (lane 4) had no effect. On the contrary, in HT29-D4 cells, no significant effect of 10 μM VLB, NOC or TAX was visible.

In HBL100 cells, the effect of drugs on c-myc protein level without EGF stimulation was evaluated (Fig. 2). We showed that 10 μM VLB compared with control (lane 5) increased c-myc 5-fold (lane 6) whilst 10 μM TAX (lane 7) had no significant effect.

We visualised the c-myc localisation by immunofluorescence (Fig. 4). We observed in HBL100, as well as in Caco-2 cells, that 10 μM VLB and 10 μM NOC increased c-myc nuclear localisation, whilst 10 μM TAX had no effect. The protein localisation remained cytoplasmic in HT29-D4 whatever the drug treatment.

3.3. *Effects of antimicrotubule agents on c-*myc *promoter activity*

To gain insight into the molecular mechanism involved in c-*myc* induction by the depolymerising agents, we transfected the different cell lines with pMpCAT DNA. After transfection, we first stimulated HBL100 cells with 10 μM VLB or 10 μM NOC and found after 48 h a 7- and 5-fold induction of CAT expression, respectively (Fig. 5). No significant variation of CAT expression was found in HT29-D4. This indicates

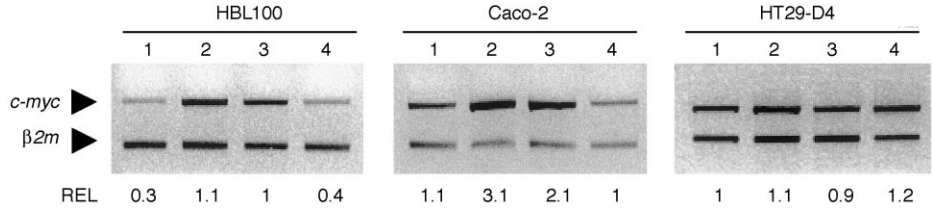

Fig. 3. Effects of antimicrotubule agents on c-*myc* expression without any prior EGF stimulation. Representative RT-PCR analysis from HBL100 cells treated for 5.5 h; Caco-2 for 7 h and HT29-D4 for 6.5 h with the different drugs. For the three cell lines, lane 1: untreated cells; lane 2: cells treated with 10 μM VLB; lane 3: cells treated with 10 μM NOC; lane 4: cells treated with 10 μM TAX.

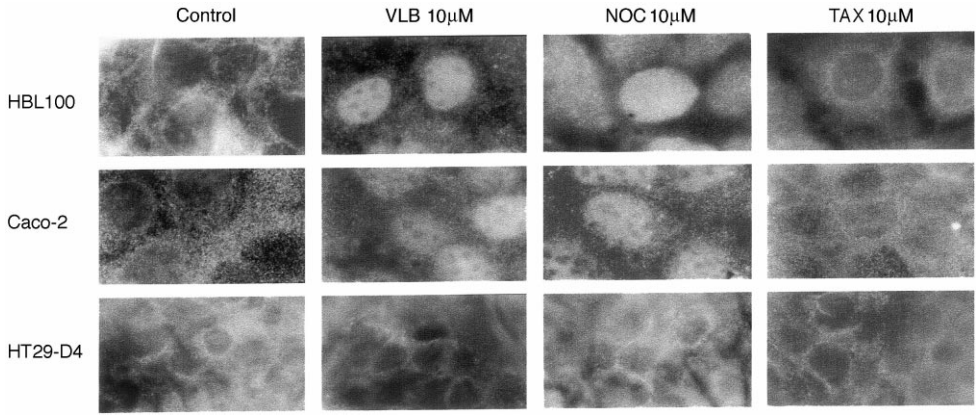

Fig. 4. Immunofluorescence showing the effects of antimicrotubule agents on c-myc localisation. Cells were treated for 7 h with the different drugs. Magnification; 100×.

that the c-*myc* induction observed (mRNA and protein) in HBL100 was actually due to the activation of gene transcription. However, we also observed in these cells a weak induction of CAT expression after treatment with 10 μM TAX. Because of an unexplainable artefactual high background of CAT expression in control transfected Caco-2 cells, we could not detect any significant variation after drug treatment.

Since we could not detect any significant CAT expression before 48 h treatment and since this CAT assay could not be performed in Caco-2 cells, we also evaluated the transcription activation by estimation of the c-*myc* hnRNA level after drug treatment. The absence of PCR amplification prior to reverse transcription (data not shown) proved that the RNA preparations were not contaminated by genomic DNA. Fig. 6 shows that, in HBL100 cells, compared with control (lane 1), 10 μM VLB (lane 2) and 10 μM NOC (lane 3) increased c-*myc* hnRNA level 4.4±1.5- and 3.7±0.7-fold, respectively, whilst 10 μM TAX (lane 4) had no significant effect (0.6±1.0). The VLB and NOC induction were decreased by α-amanitin (lanes 5 and 6). This was confirmed in Caco-2 cells, whilst in HT29-D4 no transcription activation was detected whatever the drug tested. These results suggest that c-*myc* expression increased by depolymerising agents was mediated at the transcriptional level.

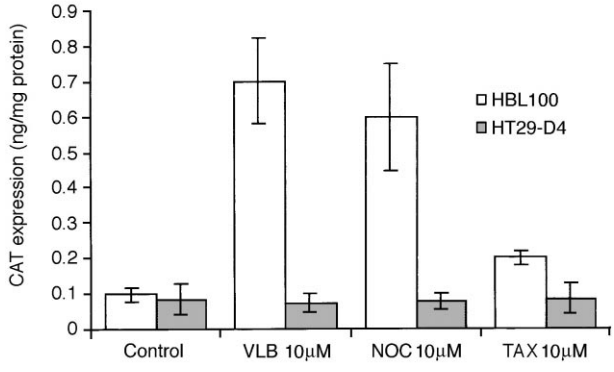

Fig. 5. Effects of antimicrotubule agents on CAT expression (CAT ELISA) in HBL100 and HT29-D4 cells. Cells were transfected by pMpCAT and stimulated for 48 h with 10 μM VLB, NOC or TAX.

4. Discussion

We showed that antimicrotubule agents (stabilising and depolymerising) decreased c-*myc* induction by EGF in HT29-D4 cells. We have studied by flow cytometry analysis (data not shown) the percentage of cells in the various phases of cell cycle during our treatment and

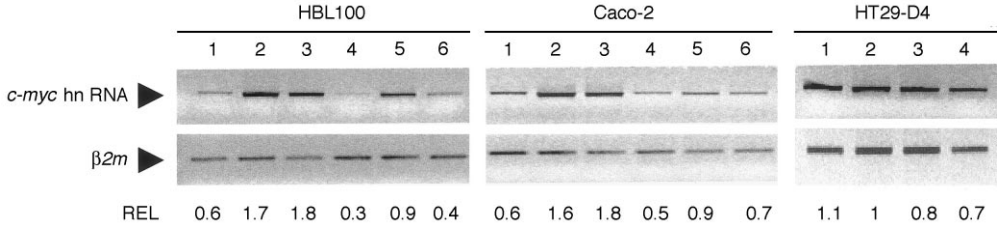

Fig. 6. Effects of antimicrotubule agents on c-*myc* hnRNA level in HBL100, Caco-2 and HT29-D4 cells. Representative RT-PCR analysis from cells treated in same conditions as in Fig. 3. Lane 1: untreated cells; lane 2: cells treated with 10 μM VLB; lane 3: cells treated with 10 μM NOC; lane 4: cells treated with 10 μM TAX; lane 5: cells treated with 10 μM VLB and α-amanitin (2 μg/ml); lane 6: cells treated with 10 μM NOC and α-amanitin (2 μg/ml).

found that less than 15% of cells were blocked in G2/M phase. So we can assume that these effects were independent of the blockage of cells as the doubling time is approximately 24 h. Moreover, treatment time was sufficient to allow drug uptake [17] but too short to induce a mitotic block. These results, obtained with EGF, are in good agreement with those found during stimulation with FBS [15]. The integrity of the microtubular network seems to be necessary in order to maintain the EGF proliferation signal, since c-*myc* induction was decreased after drug treatment, whatever the anti-microtubule agent tested (stabilising or depolymerising agents).

It was important to determine if these observations were restricted to HT29-D4 cells or if they could be extended to cells of either different (HBL100) or the same (Caco-2) tissue origin. In both these latter cell lines, the decrease in c-*myc* expression observed with 10 μM TAX could be due to the fact that the microtubules were not functional. Their dynamics was modified and consequently the c-myc signal transduction pathway and/or some intracellular transport could be disturbed. However, we observed a c-*myc* induction with high-doses of depolymerising agents in HBL100 and Caco-2 cells. Thus, Caco-2 cells do not react like HT29-D4 whilst having the same tissue origin. It seemed that HT29-D4 exhibited a particular behaviour with regard to these drugs. The induction observed with high-doses of depolymerising agents in HBL100 and Caco-2 cannot be explained by the mechanism described above. It could be inferred that in these conditions, some transduction proteins normally bound to microtubules (G proteins [22], MAPK [23], protein kinase C [24] or IκB [25]) could be released or degraded and could participate in c-*myc* induction.

Considering these results, it was important to determine if the depolymerising agents alone without any EGF treatment could have a direct effect on c-*myc* expression. In fact we observed that in HBL100 and Caco-2 cells, these agents increased the level of c-*myc* mRNA. Our data were in agreement with others who inferred that depolymerisation of microtubules with drugs could initiate DNA synthesis in the absence of other signals [26]. Moreover, stabilisation of microtubules could inhibit the action of certain mitogens [27]. It can then be concluded that disorganisation of the microtubular network modulates c-*myc* expression. Besides, VLB and NOC treatment induces translocation of c-myc protein to the nucleus. This increase in the amount of nuclear c-myc protein suggests that this transcription factor could be activated and thus could interact with its target genes.

CAT assays and hnRNA studies revealed that, in HBL100 and Caco-2 cells, depolymerising agents increased c-*myc* mRNA levels through a transcriptional activation. The transcription of the c-*myc* gene has been described as being activated via several positive regulatory elements present in its 5′ flanking region. Numerous constitutive and inducible gene regulatory proteins have been reported to govern c-*myc* expression: NFκB, E2F, Sp1, BLIMP-1/PRF and ying-yang 1 (YY1) [28], Tcf4 via adenomatous polyposis colon (APC) tumour suppressor protein [29]. An interaction has been described between tubulin and proteins implicated in the activation of these transcription factors: IκB in the case of NFκB [25], EB1 in the APC pathway [30] and that microtubule depolymerisation could activate NFκB [31]. It could then be supposed that c-*myc* induction by microtubule depolymerising agents is mediated by the release of one or several of these transcription factors.

Our conclusions were: (1) that depolymerising agents act differently on the regulation of c-*myc* expression according to the cell lines tested, probably through the presence of different transcription pathways in these cell lines; (2) that stabilising or depolymerising agents have opposite effects in the same cell line (e.g. HBL100 or Caco-2) probably via the release of protein which would act on c-*myc* promoter. This work contributes to a better understanding of microtubular network implication in c-*myc* gene regulation and signal transduction. From a pharmacological point of view, it could explain some differences in the action of antimicrotubule agents not directly linked to mitotic block. Indeed we showed that these agents, used at the same dose, could have different effects according to the cell line or tumour type. An understanding of such mechanisms could lead to a different approach combining conventional antineoplastic drugs and antisense anti-oncogene strategy.

Acknowledgements

We would like to thank Dr D. Levens for the pMpCAT plasmid; B. Charvet and A. Desobry for expert technical assistance.

References

1. Dhamodaran R, Jordan MA, Thrower D, Wilson L, Wadworth P. Vinblastin suppresses dynamics of individual microtubules in living interphase cells. *Mol Biol Cell* 1995, **6**, 1215–1229.
2. Wilson L, Jordan MA. Microtubule polymerization dynamics, mitotic block, and cell death by paclitaxel at low concentrations. *Chem Biol* 1995, **2**, 569–573.
3. Burkhart CA, Berman JW, Swindell CS, Horwitz SB. Relationship between the structure of Taxol and other taxanes on induction of TNFα gene expression and cytotoxicity. *Cancer Res* 1994, **54**, 5779–5782.
4. Bogdan C, Ding A. Taxol, a microtubule-stabilizing agent induces expression of TNFα and IL1 in macrophages. *J Leuk Biol* 1992, **52**, 119–121.
5. Bhalla K, Ibrado AM, Tourkina E, Tang C, Mahoney ME, Huang Y. Taxol induces internucleosomal DNA fragmentation

associated with programmed cell death in human myeloid leukemia cells. *Leukemia* 1993, **7**, 563–568.
6. Jordan MA, Wendell K, Gardiner S, Derry WB, Copp H, Wilson L. Mitotic block induced in HeLa cells by low concentrations of paclitaxel (Taxol) results in abnormal mitotic exit and apoptotic cell death. *Cancer Res* 1996, **56**, 816–825.
7. Donaldson J, Goolsby G, Kiener P, Wahl A. Activation of $p34^{cdc2}$ coincident with taxol-induced apoptosis. *Cell Growth Different* 1994, **5**, 1041–1050.
8. Tishler RB, Lamppu DM, Park S, Price BD. Microtubule-active drugs Taxol, Vinblastin and Nocodazole increase the levels of transcriptionally active P53. *Cancer Res* 1995, **55**, 6021–6025.
9. Bissonette RP, Echeverri F, Mahboubi A, Green DR. Apoptotic cell death induced by c-myc is inhibited by bcl-2. *Nature* 1992, **359**, 552–555.
10. Green DR. A Myc induced apoptosis pathway surfaces. *Science* 1997, **278**, 1246–1247.
11. Dooley S, Wundrack I, Blin N, Wetter C. Coexpression pattern of c-myc associated genes in a small cell lung cancer cell line with high steady state c-myc transcription. *Biochem Biophys Res Commun* 1995, **213**, 789–795.
12. Henriksson M, Luscher B. Proteins of the Myc network: essential regulators of cell growth and differentiation. *Adv Cancer Res* 1996, **68**, 109–181.
13. Hay N, Bishop JM, Levens D. Regulatory elements that modulate expression of human c-myc. *Gene Devel* 1987, **1**, 659–671.
14. Alexandrova N, Niklinski J, Bliskovsky V, *et al*. The N terminal domain of c-Myc associates with α-tubulin and microtubules *in vivo* and *in vitro*. *Mol Cell Biol* 1995, **15**, 5188–5195.
15. El Khyari S, Bourgarel V, Barra Y, Braguer D, Briand C. Pretreatment by tubulin agents decreases c-myc induction in human colon carcinoma cell line HT29-D4. *Biochem Biophys Res Commun* 1997, **231**, 751–754.
16. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guadinium thiocyanate phenol chloroform extraction. *Anal Biochem* 1987, **162**, 156–159.
17. Carles G, Braguer D, Dumontet C, *et al*. Differentiation of human colon cancer cells changes the expression of beta-tubulin isotypes and MAPs. *Br J Cancer* 1999, **80**, 1162–1168.
18. Garcia P, Braguer D, Carles G, Briand C. Simultaneous combination of microtubule depolymerizing and stabilizing agents acts at low doses. *Anticancer Drugs* 1995, **6**, 533–544.
19. Avigan MI, Strober B, Levens D. A far upstream element stimulates c-myc expression in undifferentiated leukemia cells. *J Biol Chem* 1990, **265**, 18538–18545.
20. Faure E, Leucine P, Imbert J, Champion S. Activation of the HIV type 1 long terminal repeat by X-irradiation involves two main Rel/NFκB DNA binding complexes. *AIDS Res Human Retroviruses* 1996, **12**, 1519–1527.
21. Ryu DY, Levi PE, Fernandez-Salguero P, Gonzalez FJ, Hodgson E. Piperonyl butoxide and acenaphthylene induce cytochrome P450 1A2 and 1B1 mRNA in aromatic hydrocarbon-responsive receptor knock-out mouse liver. *Mol Pharmacol* 1996, **50**, 443–446.
22. Wang N, Kun Y, Rasenick MM. Tubulin binds specifically to the signal-transducing proteins, Gsα and Giα1. *J Biol Chem* 1990, **265**, 1239–1242.
22. Reska AA, Seger R, Diltz CD, Krebs EG, Fisher EH. Association of mitogen-activated protein kinase with the microtubule cytoskeleton. *Proc Natl Acad Sci USA* 1995, **92**, 8881–8885.
24. Garcia-Rocha M, Avila J, Losano J. The ζ isozyme of proteine kinase C binds to tubulin through the pseudosubstrate domain. *Exp Cell Res* 1997, **230**, 1–8.
25. Crepieux P, Kwon H, Leclerc N, *et al*. IκBα physically interacts with a cytoskeleton-associated protein through its signal response domain. *Mol Cell Biol* 1997, **17**, 7375–7385.
26. Crossin KL, Carney DH. Evidence that microtubule depolymerization early in the cell cycle is sufficient to initiate DNA synthesis. *Cell* 1981, **23**, 61–71.
27. Crossin KL, Carney DH. Microtubule stabilization by Taxol inhibits initiation of DNA synthesis by Thrombin and by epidermal growth factor. *Cell* 1981, **27**, 341–350.
28. Levens D, Duncan RC, Tomonaga T, *et al*. DNA conformation, topology and the regulation of c-myc expression. *Curr Topics Microbiol Immunol* 1997, **224**, 33–46.
29. He TC, Sparks AB, Rogo C, *et al*. Identification of c-Myc as a target of the APC pathway. *Science* 1998, **281**, 1509–1512.
30. Morrison EE, Wardleworth BN, Askham JM, Markham AF, Meredith DM. EB1, a protein which interacts with the APC tumour suppressor, is associated with the microtubule cytoskeleton throughout the cell cycle. *Oncogene* 1998, **17**, 3471–3477.
31. Rosette C, Karin M. Cytoskeletal control of gene expression: depolymerization of microtubules activates NFκB. *J Cell Biol* 1995, **128**, 1111–1119.