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RESEARCH ARTICLE

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THE CDK1 INHIBITOR RO-3306 MODULATES THE EXPRESSION OF CELL CYCLE INHIBITORY AND APOPTOTIC GENES

ABSTRACT:

Cancer cells undergo genetic or epigenetic changes that result in uncontrolled proliferation and escape from apoptosis. Cyclin-dependent kinases (Cdks) are the master regulators of cell division. RO-3306, a selective small-molecule inhibitor of CDK1, has been shown to inhibit proliferation, induces a G2/M cell cycle arrest and apoptosis in cancer cell lines. The mechanism of action of RO-3306 involves binding to the ATP pocket of CDK1 and it also affects p53 downstream targets. The purpose of the present study was to investigate additional effects of RO-3306 through screening for changes in mRNA expression of genes known to be involved in human carcinogenesis in the human hepatocarcinoma cell line HepG2 using the RT² Profiler™ PCR Array. In the present study, RO-3306 reduced the viability, inhibited colony formation assay, induced a G2/M cell cycle phase arrest, as well as apoptosis in HepG2 cells. On the mRNA level RO-3306 increased the expression of the two cell cycle inhibitors *p21* and *p16* , as well as the expression of tumour necrosis factor (*tnf*) relative to control and it downregulated the expression of tissue inhibitor of metalloproteinase 3 (*timp3*). We confirmed the increased expression of p21 and p16 on the protein level by Western blot. Furthermore, RO-3306 induced the expression of p53 protein, while it did not alter the protein levels of CDK1 or its inhibitory phosphorylation on tryrosine 15. In conclusion, our study confirms the previous results that RO-3306 has multiple anticancer effects, and it provides further insight into the molecular events associated with these effects including the induction of the expression of cell cycle inhibitory and apoptotic genes.

KEY WORDS:

Cyclin-dependent kinase 1, RO-3306, HepG2 cells, *p53*, *p21*, apoptosis, tumour necrosis factor

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INTRODUCTION:

Targeting proteins involved in cancer development and progression is an attractive strategy for cancer therapy. Cancer cells undergo genetic or epigenetic changes that result in uncontrolled proliferation and escape from apoptosis. Cyclin-dependent kinases (Cdks) are the master regulators of cell division. Cdks are regulated through association with cyclin subunits, inhibitory and activating phosphorylation events, transcription, subcellular localization and protein degradation (Aleem and Kaldis, 2006). There are several Cdk/cyclin complexes. In contrast to Cdk2, Cdk4 and Cdk6, which are dispensable for cell cycle progression, Cdk1 is the only non-redundant Cdk (Santamaria *et al.*, 2007). It can bind cyclins A, B, E and D to drive all phases of the cell cycle (Aleem *et al.*, 2005; Santamaria *et al.*, 2007). Multi-CDK inhibitors that target CDK1 are well tolerated in cancer patients (Byrd *et al.*, 2007; Tibes *et al.*, 2008). Several potent small-molecule CDK1 inhibitors have been developed, but their activity and cell cycle profiles are not

consistent with specific CDK1 inhibition (Knockaert *et al.*, 2002; Hirai *et al.*, 2005), including flavopiridol, butyrolactone, olomoucine, and kenpaullone (Kim *et al.*, 2000).

RO-3306, a selective small-molecule inhibitor of CDK1, is a quinolinyl thiazolinone derivative, that showed good potency, selectivity towards CDK1 and a cell cycle profile (G2/M arrest), which is consistent with CDK1 inhibition (Vassilev *et al.*, 2006). RO-3306 inhibits CDK1/Cyclin B1 activity with a K_i of 35nM, nearly 10-fold selectivity relative to CDK2/Cyclin E and more than 50-fold relative to CDK4/Cyclin D (Vassilev *et al.*, 2006). The mechanism of action of RO-3306 involves binding to the ATP pocket of CDK1, acting like an ATP-competitive inhibitor leading to CDK1 inhibition, reversible G2/M arrest or apoptosis after prolonged exposure (Vassilev *et al.*, 2006). Furthermore, RO-3306 has been found to act cooperatively with the MDM2 inhibitor Nutlin-3 to induce mitochondiral apoptosis in a cell cycle-independent fashion. RO-3306 downregulates the expression of the antiapoptotic proteins Bcl-2 and survivin and blocks p53-mediated induction of p21 and MDM2 (Kojima *et al.*, 2009). The purpose of the present study was to screen for changes in mRNA expression of 84 genes, known to be involved in human carcinogenesis, induced by RO-3306 in the human hepatocarcinoma cell line HepG2. In the present work, the ability of RO-3306 to inhibit cell proliferation, to induce G2/M cell cycle arrest and apoptosis in HepG2 cells was evaluated.

MATERIAL AND METHODS:

Cell culture and drug treatment:

The HepG2 cell line was purchased from DMSZ, Germany. HepG2 cells were routinely cultured in RPMI 1640 medium supplemented with 10% foetal bovine serum (FBS) and 1% penicillin/streptomycin (Gibco, Paisley, UK). The CDK1 inhibitor R0-3306 (Calbiochem, La Jolla, CA, USA) was dissolved in dimethyl sulphoxide (DMSO) at a stock concentration of 10 mM; and was diluted to the final concentration in fresh media before each experiment.

RNA extraction and real-time quantitative PCR assay (RT^2 Profiler™ PCR Array):

RNA was extracted using the Qiagen RNeasy® Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. For RNA quality control, purity was checked by UV spectrophotometry, and ribosomal RNA band integrity was analysed by electrophoresis on 1% agarose gels. Reverse transcription reactions were prepared using the RT First Strand Kit (Qiagen, Valencia, CA, USA).

To analyse a panel of genes involved in cell transformation and tumourigenesis, RT^2 Profiler™ PCR Array: Human Cancer PathwayFinder (Qiagen, Valencia, CA, USA) was used on a ABI 7900HT 384-well block, according to the manufacturer's protocol. The Human Cancer PathwayFinder™ RT^2 Profiler™ PCR Array profiles the expression of 84 genes representative of the six biological pathways involved in transformation and tumourigenesis. Each array contains a panel of 384 primer sets for a set of cancer relevant pathways, plus five housekeeping genes and three RNA and PCR quality controls.

In brief, cDNA was prepared from 400 ng total RNA using a RT-PCR array first strand kit. Genomic DNA elimination was performed at 42°C for 5 min, and the reaction was immediately stopped by heating at 95°C for 5 min. The total volume of PCR mixture included RT qPCR master mix (SYBR Green/ROX PCR master mix), double-distilled RNAse-free H_2O, and diluted first strand cDNA synthesis reaction. 10µl per well for 384-well custom PCR array were loaded. A two-step cycling program was used for the PCR amplification reaction at the ABI 7900HT with an initial 10-min step at 95°C followed by 40 cycles of 95°C for 15 s. and 60°C for 1 min. The fluorescent signal from SYBR Green was detected immediately after the extension step of each cycle, and the cycle at which the product was first detectable was recorded as the cycle threshold. Data were generated from independent experiments and imported into an Excel database and Analysed using the comparative cycle threshold method with normalization of the raw data to housekeeping genes including β_2M, hypoxanthine phosphoribosyl transferase 1, ribosomal protein *l13a*, *gapdh*, and ^{ACTB} (β-*actin*).

Viability assay:

Cells were seeded at density of 8,000 cells in 96-well plates with a total media volume of 150 µl/well and left to grow for 24 hr. RO-3306 was then added in triplicate wells. Four hours before scoring, 10% of Alamar Blue solution (Invitrogen AB, Sweden) diluted in culture medium was added and the absorbance was measured at 550/590 nm using a microplate reader (Wallac, Turku, Finland).

Focus formation assay:

Cells were seeded in 10 cm Petri-dishes at low densities (10,000 cells per dish) and left to grow for 24 hr then treated with 5 µM of RO-3306 and left to grow for 2 weeks. The dishes were then washed twice with cold phosphate-buffered saline (PBS) (Hyclone, logan, UT) then fixed with cold methanol for 10 min, stained with 0.5% of crystal violet dye (Sigma, St Louis, MO, USA) for 10 min at room temperature (RT). The dishes were then rinsed with tap water and left to dry. The number of colonies in each plate was counted.

Cell cycle and flow cytometry:

Analysis of cell-cycle distribution and percentage of cells in S phase was measured by the incorporation of bromodeoxyuridine (BrdU; Sigma, St Louis, MO, USA) into newly synthesized DNA, followed by propidium iodide (PI) staining. Cells were pulse-labelled with 100 µM BrdU for 1 hr before the indicated time points and then harvested. For flow cytometry, cells were washed with PBS, fixed with 70% ethanol overnight. Cells were then treated with 0.01% RNase then permeabilized with 0.05% pepsin and 2N HCl, followed by staining with mouse anti-BrdU antibody (Becton Dickinson, San Jose, CA) for 1hr at RT. After washing, cells were stained with anti-mouse IgG FITC-conjugated secondary antibody (DAKO) for 45 min at RT, followed by PI staining (25 µg/ml) for 1hr at RT. The samples were then analysed by flow cytometry using a FACSCalibur (Becton Dickinson), and the data were processed with the Cell Quest software (Becton Dickinson).

Annexin V/PI assay:

Apoptosis was studied using Annexin V/PI method (Annexin V-FLUOS staining kit, Roche, Mannheim, Germany) according to the manufacturer's protocol. Briefly, cells were treated with RO-3306 for 48 hrs. $1x10^6$ cells were washed with ice-cold PBS without Ca^{+2}or Mg^{+2} (Gibco, UK). The cells were then resuspended in 100 µl of labelling solution and incubated with 0.1µg/ml PI and 2 µl Annexin V-fluorescein for 15 min in the dark at RT. Flow cytometric analysis was immediately performed using FACSCalibur (Becton Dickinson).

Preparation of lysates:

Cells were seeded in 10 cm dishes. After 24 hr cells were treated with RO-3306 for 24 hr. Cells were then harvested and washed twice with cold PBS, then lysed in Modified Radioimmunopreciptation lysis buffer (RIPA) buffer; [0.05 M Tris.HCl (pH 7.5), 1% NP40, 0.25% Sodium Deoxycholate, 150 mM NaCl, 1 mM EDTA, NaF, protease inhibitor cocktail (Roche, Mannheim, Germany), and phosphatase inhibitors 1 and 2 (Sigma, St Louis, MO, USA)]. Lysates were centrifuged for 30 min at 16,000 *x g* at 4ºC, supernatents transferred into fresh tubes and frozen at -80ºC until use. Protein concentrations were determined using the BCA protein assay (Pierce Biotechnology, Rockford, IL, USA) according to manufacturer's protocol.

Western blot:

Samples corresponding to 15 µg of total protein were resolved on NuPage 4-12% Bis-Tris SDS/PAGE gels (Invitrogen, Carlsbad, CA, USA). Molecular weight markers from Bio-Rad (Bio-Rad, Hercules, Ca) and Invitrogen (Invitrogen, Carlsbad, Ca) were run simultaneously. The proteins were transferred onto nitrocellulose membranes (Hybond, Amersham, UK) and blocked with either 5% milk or bovine serum albumin (BSA) in a Tris-Buffered Saline and Tween 20 buffer (TBST) [19.97 mM Tris base, 135 mM NaCl, 0.1% Tween 20] and incubated with the following primary antibodies: rabbit anti-phospho-tyrosine15 Cdk1 (Cell Signalling Technology, Beverly, MA, USA), rabbit anti-p16 (Cell Signalling Technology, Beverly, MA, USA), mouse anti-p21 (Cell Signalling Technology, Beverly, MA, USA), mouse anti-p53 (Becton Dickinson, San Jose, CA, USA) and rabbit anti-Cdk1(Calbiochem, La Jolla, CA, USA). Membranes were then washed in TBST and incubated with either anti-rabbit or anti-mouse IgG horseshoe peroxidase (HRP)-conjugated secondary antibody (Amersham, Buckinghamshine , UK) and protein bands detected with enhanced chemiluminescence (Amersham, Buckinghamshine, UK).

Statistics:

Experiments were performed in triplicates and data were expressed as mean ± standard deviation.

RESULTS:

RO-3306 reduces viability and inhibits colony formation:

We studied the effect of RO-3306 on HepG2 cell viability using two different concentrations (0.5 and 5 µM). The low concentration did not show significant effect on HepG2 cells even after 5 days of treatment. The higher dose (5 µM) reduced viability of HepG2 cells significantly. After 24 hr of treatment the surviving fraction was 51%, which further decreased to only 20% after 5 days of treatment (Fig. 1A). Furthermore, 5 µM RO-3306 inhibited colony formation in HepG2 cells, compared to untreated cells (Fig. 1B).

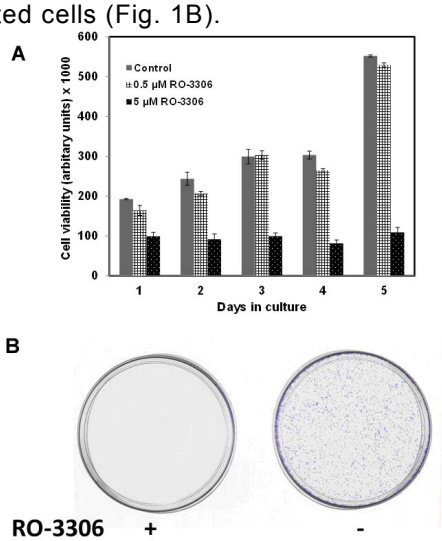

Fig. 1. RO-3306 reduces viability and inhibits colony formation in HepG2 cells. Treatment of HepG2 cells with RO-3306 markedly reduced viability of HepG2 cells at the 5µM concentration in comparison to control cells (DMSO-treated) (A). Data are shown as mean of triplicate experiments ± standard deviation. Treatment of the cells with 5 µM RO-3306 inhibited colony formation (B).

RO-3306 induces cell cycle arrest at the G2/M phase:

Cell cycle analysis was performed using PI and BrdU staining. Treatment of HepG2 cells with 5 µM RO-3306 for 24 hr. resulted in significant G2/M phase arrest reaching almost 5-fold that of untreated cells (Fig. 2 A&B). This was accompanied by a decrease in the percentage of cells in G1 and S-phase by almost 50 % (Fig. 2 A&B). Thus, RO-3306 arrests cells in the G2/M phase without an effect on S phase progression.

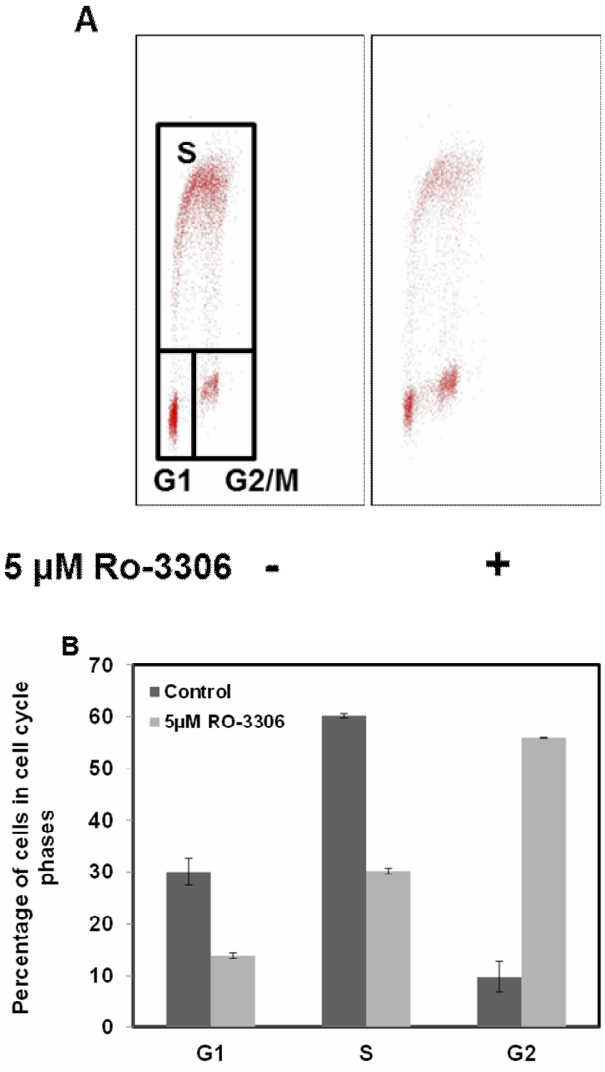

Fig. 2. RO-3306 induces G2/M arrest. HepG2 cells were treated with either DMSO (control) or 5µM RO-3306 for 24 hr, then analysed for cell-cycle distribution using pulse labelling with BrdU followed by staining with anti-BrdU antibody and PI and FACS analysis (A). RO-3306 induced a 5-fold increase in the population of cells in G2/M phase (B).

RO-3306 induces apoptosis:

Treatment of HepG2 cells with 5 µM RO-3306 for 48 hr resulted in 70% increase in apoptotic cells in comparison to control cells (DMSO-treated) using Annexin V/PI assay (Fig. 3). Apoptotic cells refer to the total of early and late apoptosis.

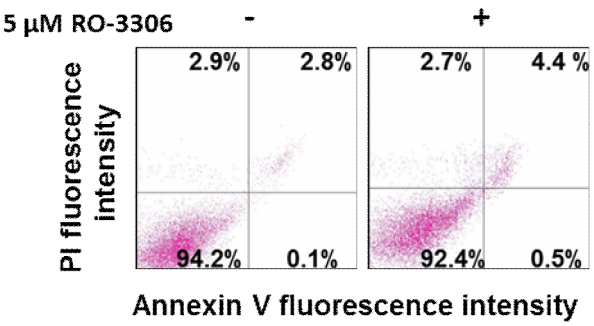

Fig. 3. RO-3306 induces apoptosis. HepG2 cells were treated with either DMSO (control) or 5 µM RO-3306 for 48 hr, then analysed for apoptosis using Annexin V/PI method followed by FACS analysis. RO-3306 induced about 70% increase in the percentage of apoptotic cells (early+late apoptosis) in comparison to control.

RO-3306 modulates the mRNA expression of genes in four carcinogenesis pathways:

In order to investigate the molecular mechanism of action of RO-3306 in HepG2 cells, RT-PCR arrays were used to compare the mRNA levels of different genes involved in carcinogenesis in the treated cells versus those of the control cells (Table 1, Fig. 4). RT PCR arrays measure the expression levels of numerous genes simultaneously, thus dramatically accelerating the time of investigation (Fig. 4 A&B). Using the Human Cancer PathwayFinder™ RT-PCR array, we screened the effect of RO-3306 treatment on 84 genes regulating different pathways in cancer. The 84 genes were grouped according to their function into the following pathways: Cell cycle and DNA damage repair, apoptosis and cell senescence, signal transduction molecules and transcription factors, adhesion, angiogenesis, and invasion and metastasis. In the present study treatment of HepG2 cells with 5µM RO-3306 resulted in statistically significant changes in the mRNA expression of a number of genes categorized into four cancer-relevant pathways (five selected genes are shown in table 1). RO-3306 increased the mRNA expression of the two cell cycle inhibitors $p21^{WAF1/CIP1}$ and $p16^{INK4A}$ by 1.7 fold and 1.4 folds, respectively in comparison to control (Fig. 4C). Although this fold change was not pronounced but it was also confirmed on the protein level. Furthermore, RO-3306 increased the expression of tumour necrosis factor (*tnf*) by almost 2.5 folds relative to control. In the present study, RO-3306 downregulated the expression of tissue inhibitor of metalloproteinase 3 (*timp3*). In addition, treatment with RO-3306 resulted in an increase of 1.6 fold in the expression of the breast cancer specific gene-1 (*bcsg-1/gamma-synuclein*) also known as *sncg* and the expression of the ribosomal protein *l13a* (*rpl13a*) (Table 1, Fig. 4C).

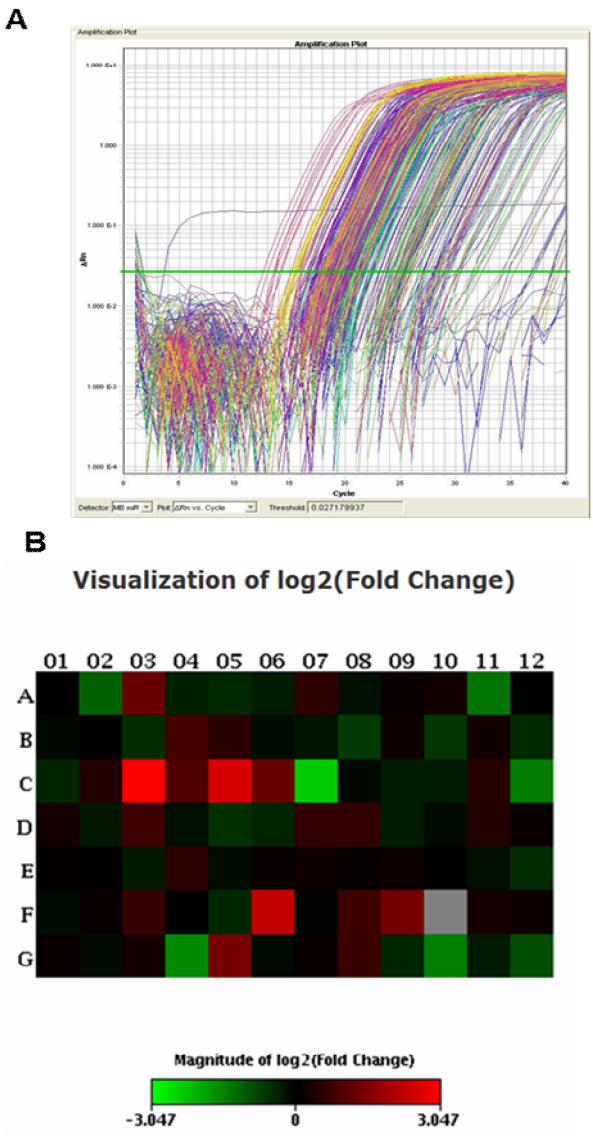

Fig. 4. RO-336 modulates the expression of genes relevant to carcinogenesis. Amplification plot of real-time RT-PCR of 84 genes showing threshold (green horizontal line) and amplification profiles (A). Results of the Human Cancer PathwayFinder™ array is presented as a heat map (B). Colour designation: Black: a median expression value equals to one; red: increased expression; green: reduced expression; grey: undetectable expression. The changes in expression of 10 genes by RO-3306 as fold difference relative to control cells (C). Data are expressed as mean and standard deviation.

Table 1. Effect of RO--3306 on expression of selected cancer-relevant genes

Cancer pathway	Gene	Description*	Fold change relative to control
Cell Cycle and DNA repair	CDKN1A	cyclin-dependent kinase inhibitor 1A (*p21*, Cip1)	1.7
	CDKN2A	cyclin-dependent kinase inhibitor 2A (p16)	1.4
Signal transduction molecules and transcription factors	SNCG	synuclein, gamma (breast cancer-specific protein 1)	1.6
Apoptosis	TNF	Tumour necrosis factor	2.6
Invasion and metastasis	TIMP3	Tissue inhibitor of metalloproteinases 3	0.3

*From Gene cards. www.genecards.org

RO-3306 induces the expression of p53, p21 and p16:

Next we confirmed that the RO-3306-induced mRNA expression of *p21* and *p16* could be detected on the protein level using Western Blots (Fig. 5). Furthermore, we studied the protein levels of cell cycle regulatory proteins involved in G2/M progression. In addition to p21 and p16 RO-3306 induced the accumulation of p53 while it had no effect on the inhibitory phosphorylation at tyrosine 15 of CDK1 or on the levels of CDK1 protein (Fig. 5).

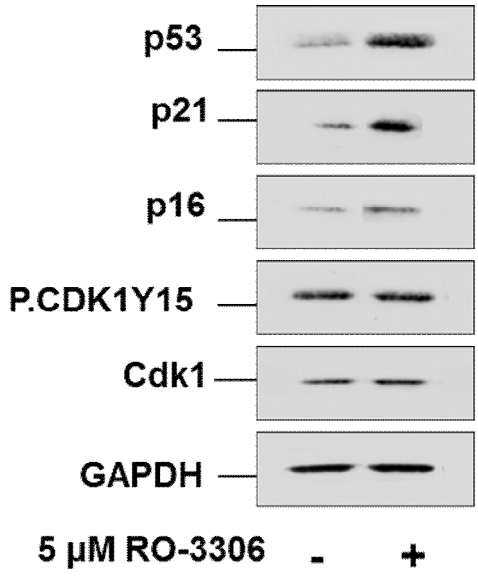

Fig. 5. RO-3306 induces the accumulation of cell cycle inhibitors. RO-3306 induced the expression of the cell cycle inhibitors p53, p21 and p16, without affecting the tyrosine 15 (Y15) phosphorylation levels or total protein levels of CDK1. GAPDH was used as loading control.

DISCUSSION:

The efficacy and selectivity of RO-3306 towards CDK1 inhibition have been reported in a number of cell lines including colon- , and cervical cancer cell lines (Vassilev *et al.*, 2006). RO-3306 reversibly arrests human cells at the G2/M border of the cell cycle in normal human cells while it also induces apoptosis in tumour cells (Kojima *et al.*, 2009). It has been suggested that RO-3306 enhances downstream signalling of p53 to promote apoptosis (Kojima *et al.*, 2009). In the present study, RO-3306 significantly reduced cell viability and colony formation in HepG2 cells, arrested the cells at the G2/M phase of the cell cycle and induced apoptosis. In order to understand the molecular events associated with cell cycle arrest and apoptosis induced by RO-3306 we studied the changes in gene expression of 84 genes relevant to transformation and tumourigenesis using real-time quantitative RT-PCR arrays. This primary screening revealed that treatment of HepG2 cells with RO-3306 induced the expression of two cell cycle inhibitory genes; *p21* and *p16*, and this expression was further confirmed on the protein level using Western blot. RO-3306 also induced the accumulation of p53 on the protein level. The tumour-suppressor p53 functions to maintain the integrity of the genome and is often activated in response to a variety of stress signals including DNA damage, oncogene activation and hypoxia, resulting in either cell cycle arrest or apoptosis (Giaccia and Kastan, 1998; Slee *et al.*, 2004). *p53* is also induced by chemotherapeutic agents in wild-type p53 containing tumours and plays a crucial role in controlling the proliferation of these tumours (Lowe *et al.*, 1994; El-Deiry, 2003). It is noteworthy to mention that HepG2 cells used in the present study express wild-type p53. Therefore, the induction of p53 by RO-3306 is an important molecular mechanism mediating both cell cycle arrest and apoptosis in HepG2 cells. $p21^{WAF1/CIP1}$ is the main downstream effector of p53's growth arrest function (el-Deiry *et al.*, 1993; Gartel *et al.*, 1996; Gartel and Tyner, 1999). Thus, RO-3306 may cause G2/M arrest through upregulation of p53 levels, which in turn causes enhanced transcription of the CDK1 inhibitor p21. In contrast to our study, treatment of the acute myeloid leukemia cell line (AML) with RO-3306 was not associated with p53 induction (Kojima *et al.*, 2009). This discrepancy could be explained by the fact that the duration of RO-3306 treatment was for 6 hr in Kojima *et al*. (2009), while in our study we treated the HepG2 cells for 24 hr before we observed the induction of p53. This is consistent with a previous study, which reported that RO-3306-induced G2/M arrest occurs after 20 hr of treatment (Vassilev *et al.*, 2006).

In the present study RO-3306 neither had an effect on the tyrosine 15 phosphorylation of CDK1 (which is phosphorylated by Wee1/Myt1 and dephosphorylated by Cdc25 family of phosphatases) nor on CDK1 protein levels indicating that this pathway of CDK1 regulation (Checkpoint kinases-Cdc25 phosphatases) is not affected by RO-3306.

In the present study, RO-3306 induced apoptosis in HepG2 cells after 48 hr. This is in agreement with previous studies in colon cancer cells (Vassilev *et al.*, 2006). Kojima *et al.* (2009) showed that the mechanism of RO-3306-induced apoptosis involves downregulation of survivin and Bcl-2. In the present study, we have not observed changes in Bcl2 or survivin, however a 2.6-fold increase in the mRNA expression level of the tumour necrosis factor (*tnf*) was detected. *tnf* encodes a multifunctional proinflammatory cytokine, which is mainly secreted by macrophages. It is involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation. This cytokine has been implicated in a variety of diseases including cancer.

Among the cytokines expressed by HepG2, Stonans *et al.* (1999) have determined by RT-PCR the capacity of HepG2 cells to express mRNAs for *ifn-gamma* and *tnf-alpha* among other cytokines. Out of the members of the *tnf* superfamily, *tnf* is probably the most potent inducer of apoptosis. *tnf* activates both cell-survival and cell-death mechanisms simultaneously. Activation of *nf-kβ* -dependent genes regulates the survival and proliferative effects of *tnf*, whereas activation of caspases regulates the apoptotic effects (Rath and Aggarwal, 1999). In the present study, RO-3306 did not affect the gene expression of *nf-kβ*. It remains to be elucidated whether *tnf* induced by RO-3306 plays a role in RO-3306-induced apoptosis.

Furthermore, in the present study RO-3306 reduced the expression of the tissue inhibitor of metaloproteinases-3 (*timp-3*). The proteins encoded by the *timp* gene family are inhibitors of the matrix metalloproteinases, a group of peptidases involved in degradation of the extracellular matrix (Djuranovic *et al.* 2006). Expression of this gene is induced in response to mitogenic stimulation. Upregulation of *timp-3* expression in HCC-7721 cells inhibits invasion capacity *in vitro*, as well as tumourigenic and metastatic potential in nude mice (Zhang *et al.*, 2007). Furthermore, induction of G1 phase arrest in HCC cells by APMCF1 was associated with downregulation of *timp-3* and upregulation of *p21* (Li *et al.*, 2006). In agreement with this study, our results also show downregulation

of *timp-3* and upregulation of *p21* associated with RO-3306-induced G2/M arrest.

Surprisingly, RO-3306 induced the expression of synuclein, gamma (breast cancer-specific protein 1) (*sncg*) also known as *bcsg1*. *sncg* is highly expressed in the advanced staged breast carcinoma, in addition to a number of other tumours (Bruening *et al.*, 2000; Ji *et al.*, 1997). Several lines of evidence suggest that *bcsg1* plays a positive role in the process of invasion and metastasis of breast cancer cells. *In vitro* studies demonstrate that ectopic expression of *bcsg1* in breast cancer cells significantly stimulates cell proliferation and cell migration (Jia *et al.*, 1999). These data suggest that RO-3306 may enhance invasion potential of HepG2 cells. This result warrants further investigation. In this context, some endogenous proteins have dual contradictory functions such as $p27^{kip1}$, which has been identified as a tumour suppressor with an essential cell cycle inhibitory function (Polyak *et al.*, 1994; Fero *et al.*, 1996; Kiyokawa *et al.*, 1996; Nakayama *et al.*, 1996) but it has also been demonstrated that *p27* may function as an oncogene through promoting cell migration (Besson *et al.*, 2004).

In conclusion, our study using HepG2 cells confirmed the previous results that RO-3306 has multiple anticancer effects. It inhibited colony formation, proliferation and induced G2/M arrest and apoptosis in HepG2 cells. Furthermore, RO-3306 treatment increased the mRNA expression of the two cell cycle inhibitors *p21* and *p16*, and the tumour necrosis factor (*tnf*), as well as increased the protein levels of p53 and *bcsg1* relative to control. However, it downregulated the expression of the tissue inhibitor of metalloproteinase 3 (*timp3*). Results from the present study provide further insight into the molecular events associated with RO-3306 effects including the induction of the expression of cell cycle inhibitory and apoptotic genes.

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عقار RO-3306 المثبط لنشاط CDK1 يغير في تعبير الجينات المثبطة لدورة الخلية و المحثة للموت المبرمج

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تحدث بالخلايا السرطانية تغيرات وراثية و فوق وراثية (epigenetic) تؤدي إلى التكاثر غير المنظم والهروب من موت الخلايا المبرمج.انزيمات الكينسات المعتمدة على السيكلين (cyclin) (Cdks) هي المتحكم الرئيسي في عملية انقسام الخلية. وجد أن RO-3306 و هو جزيء صغير مثبط لنشاط CDK1 يعوق تكاثر الخلايا و يحث على تراكمها في مرحلة G2 / M من دورة الخلية وكذلك على الموت المبرمج و ذلك فى العديد من خطوط الخلايا السرطانية. آلية عمل عقار RO-3306 تشمل الارتباط بجيب الATP لCDK1 كما انه أيضا يؤثر في البروتينات المستهدفة بال p53. كان الهدف من هذا البحث هو دراسة تأثيرات أخرى لل RO-3306 من خلال مسح شامل للتغيرات في تعبير الجينات التي تلعب دورا في عملية التسرطن فى الإنسان في خط خلايا سرطان الكبد المعروف بال HepG2 و ذلك باستخدام مصفوفة ال RT^{2}Profiler^{TM} PCR Array. من خلال هذه الدراسة وجدنا أن RO-3306 يثبط تكاثر الخلايا و يحول دون انتشار وتكوين مستعمرات خلوية و إلى تراكم الخلايا في مرحلة G2 / M من دورة الخلية و إلى موت الخلايا المبرمج. نتج من المعالجة بال RO-3306 زيادة في التعبير الجيني لل p21 وp16 و كذلك لل TNFبالمقارنة بالخلايا الغير معالجة كما أدى إلى نقصان في التعبير الجينى لTIMP3. و قد تأكدنا أيضا ان الزيادة المستحدثة فى تعبير ال p21وp16 الجيني تقع أيضا على مستوى البروتين و ذلك باستخدام تقنية Western blot. كما أدى العقار إلى زيادة في التعبير البروتينى لل p53 و لم يؤثر على مستوى CDK1 أو على مقدار الفسفرة المثبطة لل CDK1 على تيروسين 15. النتائج المستنبطة من هذا البحث تؤكد النتائج السابقة أن عقار RO-3306 له العديد من التأثيرات المضادة للأورام كما تلقى نتائج البحث الضوء على المزيد من الآلية الجزيئية لتأثير RO-3306 مثل استحداث التعبير في الجينات المثبطة لنشاط دورة الخلية و كذلك المستحثة للموت المبرمج.

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