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Differential expression of the *RTP/Drg1/Ndr1* gene product in proliferating and growth arrested cells

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Abstract

Using a differential display method to identify differentiation-related genes in human myelomonocytic U937 cells, we cloned the cDNA of a gene identical to *Drg1* and homologous to other recently discovered genes, respectively human *RTP* and *Cap43* and mouse *Ndr1* and *TDD5* genes. Their open reading frames encode proteins highly conserved between mouse and man but which do not share homology with other know proteins. Conditions in which mRNAs are up-regulated suggest a role for the protein in cell growth arrest and terminal differentiation. We raised antibodies against a synthetic peptide reproducing a characteristic sequence of the putative polypeptide chain. These antibodies revealed a protein with the expected 43 kDa molecular mass, up-regulated by phorbol ester, retinoids and 1,25-$(OH)_2$ vitamin D_3 in U937 cells. It was increased in mammary carcinoma MCF-7 cells treated by retinoids and by the anti-estrogen ICI 182,780 but not by 4-hydroxytamoxifen. The mouse Drg1 homologous protein was up-regulated by retinoic acid in C2 myogenic cells. The diversity of situations in which expression of *RTP/Drg1/Ndr1* has now been observed shows that it is widely distributed and up-regulated by various agents. Here we show that ligands of nuclear transcription factors involved in cell differentiation are among the inducers of this novel protein. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Drg1; mRNA differential display; Differential expression; Retinoid; Vitamin D_3; Estrogen

1. Introduction

The progression of cells towards terminal differentiation requires the concerted expression of numerous specific genes. Established cell lines allow to reproduce in vitro some aspects of this process and provide experimental models to investigate the molecular mechanisms through which transformed cells recover the phenotype of their normally differentiated counterparts. Human leukemic U937 cells cease to proliferate and express functional properties upon treatment with 1,25-$(OH)_2$ vitamin D_3 (VD) or retinoic acid (RA). We have previously shown that these agents and their derivatives act synergistically to induce the macrophagic differentiation of U937 cells, the highest effect being achieved with combinations of VDR, RXR and RAR agonists ([1] and references therein). We used a strategy based on the differential display technique of Liang and Pardee [2] to identify novel genes expressed in differentiating U937 cells. Among a collection of differentially expressed fragments of cDNA, we focused our attention on a

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strongly induced cDNA which proved to be identical to Drg1, recently cloned in colon epithelial cells [3] and, with one nucleotide difference, similar to RTP and Cap43, discovered by differential display in other experimental models [4,5]. Comparison of the potential mouse and human open reading frames indicates a protein highly conserved between mammalian species. However, these sequences do not reveal any homology with other known proteins, susceptible to give indications on the role of the protein in cell physiology. The fact that its mRNA is up-regulated under conditions where cells cease to proliferate suggests a role for the protein in cells undergoing terminal differentiation. In order to study *RTP/Drg1/Ndr1* expression at the protein level, we raised antibodies against a repeated sequence characteristic of the putative polypeptide chain. Western blot analyses revealed a 43 kDa protein, consistent with the predicted open reading frame, which actually displayed increased expression in various models of human and mouse cells induced to growth arrest and differentiation.

2. Materials and methods

2.1. Cell lines

The U937 (myelomonocytic cell line, human; cell culture conditions described in [1]), Jurkat (leukemic-T cell line, human; cell culture conditions described in [6]) and MCF-7 (mammary carcinoma cell line, human; cell culture conditions described in [7]) were purchased from ATCC (Rockville, MD, USA). Mouse C2 myogenic cells and the C2-R subclone were a generous gift from Dr. A. Bonnieu (cell culture conditions described in [8]).

2.2. mRNA differential display and multiplex messenger assay

Total RNA extracted from U937 cells with Trizol (Gibco BRL, Life Technologies, Gaithersburg, MD, USA) and cleaned up with DNase I was used for reverse transcription with Superscript II reverse transcriptase (Gibco BRL). For differential display, PCR reactions were performed using RNA Image following manufacturer's instructions (GenHunter, Nashville, TN, USA), with random 30-mers and anchored primers. Amplified cDNA fragments, labeled by incorporation of [α-^{32}P]dCTP, were separated on 6% polyacrylamide gels. Following electrophoresis, the gels were layered on pieces of 3M paper without drying and radioactivity measured using a Storm Analyser (Molecular Dynamics, Sunnyvale, CA, USA). The bands of interest were cut from the gel, eluted and re-amplified using the same PCR conditions. Each selected cDNA fragment was cloned into a pCR-TRAP vector (GenHunter) according to manufacturer's instructions. Plasmids were extracted with the Qiagen Plasmid Mini Kit (Qiagen, Courteboeuf, France). The plasmids were dissolved in water, boiled for 10 min, and cooled. Three different amounts of each plasmid in 2×standard saline citrate (SSC), respectively 75, 150 and 300 ng, were spotted in duplicate on Hybond-N nylon membranes (Amersham Life Science, Buckinghamshire, UK) briefly wetted in 2×SSC. Dot blotting was performed using a Bio-Rad manifold (Bio-Rad, Ivry, France). Following application of the plasmids, membranes were rinsed twice with 500 μl of 2×SSC, removed from the manifold and dried for 1 h at 80°C in a vacuum oven. Filters were prehybridized for 4 h with 50% formamide, 5×SSPE, 5× Denhardt, 1% SDS, 20 μg/ml salmon sperm DNA and 5% dextran at 42°C. Two probes were prepared, starting from 5 μg of total RNA extracted respectively from proliferating and 4 h differentiated U937 cells. The samples were dissolved in 12 μl DEPC-treated H_2O with 1 μl of oligo(dT_{12-18}) (500 μg/ml), heated for 10 min at 70°C and quickly chilled on ice. After a brief centrifugation, 1 μl of (dATP, dGTP and dTTP (25 μM each), dCTP (0.25 μM)), 2 μl of DTT (0.1 M) and 5 μl of [α-^{32}P]dCTP (6000 Ci/mmol) were added and the mixture incubated for 2 min at 42°C. Superscript II reverse transcriptase (Gibco BRL) was then added and the reaction performed for 1 h at 42°C. The radioactive cDNA was isolated by filtration through a Sephadex G-25 column eluted with Tris 50 mM, LiCl 0.4 M. A sample containing 10 million cpm was boiled for 10 min, chilled on ice and added to the prehybridization mixture. Hybridization was performed overnight, and membranes washed in 0.2×SSC at 60°C. The amounts of radioactivity retained on filters were evaluated using a Storm Phosphor Imager (Molecu-

lar Dynamics). Spots were visualized and the radioactivity was quantified using the Storm Imager software. Standardization was based on the signal given by β_2-microglobulin mRNA.

2.3. 5′-RACE

Total RNA was extracted with Trizol (Gibco BRL Life Technologies). The 5′-RACE reaction was performed using 5′-RACE for Rapid Amplification of cDNA Ends, version 2.0 according to the manufacturer's protocol (Gibco BRL). The primers were as follows: for the first strand cDNA synthesis: 5′-TAA-GAA-ACT-CCT-CTG-G-3′ and for PCR: 5′-AGT-CAT-TGC-CTC-TCA-CG-3′.

2.4. Northern blot analyses

Total RNAs extracted from cultured cells were separated by electrophoresis in 1% agarose/formaldehyde denaturing gel and transferred onto nylon membranes (Hybond-N, Amersham). cDNA probes were labeled with [^{32}P]dCTP using a random priming kit (Boehringer Mannheim). Hybridization was performed at 42°C in 50% formamide, and membrane washes were done in 0.2×SSC (60°C). The amounts of probe retained on filters were measured in situ using a Storm Phosphor Imager (Molecular Dynamics).

2.5. Immunological detection of the Drg1 protein

Rabbit polyclonal antisera were raised against $(EGTRSRSHTS)_2$. Synthesis of the peptide and rabbit immunizations was performed by AGRO-BIO (Villeny, France). For Western blotting, cells were lysed in 4×sample buffer (10% Tris 1 M pH 6.8, 30% SDS 20%, 30% glycerol, 30% β-mercaptoethanol). Cell extracts (each corresponding to 100 000 cells/lane) were fractionated by SDS-PAGE on a 10% polyacrylamide gel and blotted onto nitrocellulose filters using a semi-dry electroblotter (Millipore, St Quentin en Yvelines, France) at 0.8 mA/cm^2 for 1 h. The nitrocellulose filters were then blocked with 5% non-fat powdered milk in PBS and incubated with the antisera diluted in PBS. The filters were probed with mouse peroxidase labeled protein A and the specific complexes were revealed by ECL-photochemiluminescence according to the manufacturer's protocol (Amersham, Les Ullis, France). For detection of Drg1 by immunofluorescence, cells were fixed in cold methanol for 2 min and permeabilized with 0.1% Triton X-100 in PBS for 5 min. Cells were incubated with AB human serum (HS) for 10 min, washed with PBS, incubated for 2 h with anti-Drg1 polyclonal antibodies (1/250 dilution in PBS with 1% HS), washed twice, incubated with rhodamine goat anti-rabbit antibody for 1 h and rinsed twice in PBS. Fluorescence was observed with a Leica confocal microscope. For flow cytometry analyses, cells were fixed in suspension with 1% paraformaldehyde in PBS/Tween 0.01% v/w for 2 h and permeabilized with PBS/Tween 0.5%/BSA 2% for 10 min. Cells were preincubated for 10 min with HS, for 1 h with rabbit polyclonal antibodies, washed 3 times, incubated for 30 min with fluorescein-conjugated secondary antibody and washed 3 times in PBS. Cell fluorescence intensity was measured using a FACS-Calibur analyzer (Becton Dickinson) and data processed using manufacturer's programs.

3. Results

3.1. Detection and characterization of Drg1 in U937 cells by mRNA differential display analysis

We used a mRNA differential display assay to compare gene expression in proliferating U937 cells and in cells treated for 4 h with VD associated with a combination of RAR and RXR agonists, respectively TTNPB and LGD1069 [1]. In a series of experiments using different combinations of primers, about 1500 bands were visualized on gel autoradiograms and 42 of them were found to display significant variations. The corresponding cDNA fragments were eluted, cloned and spotted on filters for multiplex messenger assay (MMA) [9]. Data were further re-evaluated by Northern blotting [10]. Fig. 1 shows the visual patterns (A,C) and quantitative data (B) for several clones, selected to illustrate the various situations encountered in this analysis.

Interestingly, clone 26 was strongly up-regulated during U937 cell differentiation, as confirmed by MMA and Northern blot (Fig. 1). Sequencing of this cDNA fragment (303 bp) and computer data-

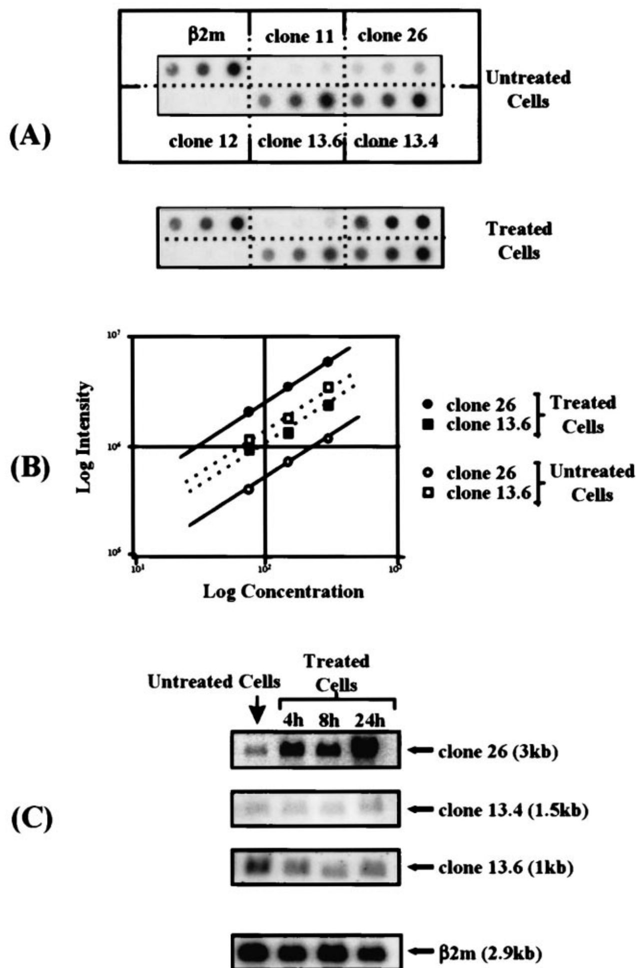

Fig. 1. Multiplex messenger assay and Northern blot analyses. (A) 75, 150 and 300 ng of plasmids were dotted in duplicate sets onto nylon membranes and hybridized with ^{32}P labeled cDNA prepared by reverse transcription of mRNAs extracted respectively from proliferating U937 cells (untreated cells) and from U937 incubated for 4 h with 1,25-dihydroxy vitamin D_3 (100 nM), TTNPB (100 nM) and LGD1069 (1 μM) (treated cells). A human β_2-microglobulin (β2m) probe, cloned in a pCR-TRAP vector, was used for quantitative calibration. (B) Comparison of intensity values after hybridization of filters described in A. (C) Northern blots were carried out with 30 μg of total RNA from untreated or treated cells as indicated and hybridized with ^{32}P labeled plasmids as indicated.

base search indicated 100% homology to the differentiation-related gene (*Drg1*) cDNA, previously reported by van Belzen et al. [3]. 5′-RACE allowed to obtain the full coding sequence (1184 bp), confirming that the cDNA identified in U937 was identical to *Drg1* (GenBank accession No. X92845) and, with one amino acid difference, homologous to the two human genes *RTP* (99.5% homology; GenBank accession No. D87953) and *Cap43* (99.5% homology; GenBank accession No. AF004162). These genes are homologous to two *Mus musculus* genes, respectively *Ndr1* (83.5% homology; GenBank accession No. U60593) and *TDD5* (82.5% homology; GenBank accession No. U52073). Sequence alignments are given elsewhere [5]. The mammalian genes are slightly homologous to the *Caenorhabditis elegans* gene ZK1073.1 (29% homology; GenBank accession No. Z68135).

3.2. Immunological characterization of the putative Drg1 protein

The open reading frame predicts a 394 amino acid peptide chain, with a deduced molecular mass of 43 kDa. Computer database searches using a variety of programs available on the Infobiogen site (http://infobiogen.fr) have not until now revealed significant consensus motifs susceptible to give indications about its biological function, except for a possible phosphopantethein attachment site identified with PROSITE (http://genebio.com/prosite.html). The most prominent feature is a novel highly hydrophilic ten amino acid unit tandemly repeated three times near the C-terminus.

Since this sequence was susceptible to provide an epitope for the immunological detection of the protein, we selected within it a ten amino acid tandem repeat, allowing to design a 20-mer synthetic peptide which was used as immunogen. This sequence is present in the human RTP, Drg1, Cap43 and, with minor differences, in the mouse Ndr1 and TDD5 proteins (Fig. 2). High titer rabbit polyclonal antibodies were obtained, recognizing one band with the expected 43 kDa molecular mass (Fig. 3A) in Western blot assays of U937 cell extracts: an ECL signal was recorded in less than 1 min using a 5000-fold dilution of the antiserum sampled on day 53 after the first injection and two booster injections. No signal was observed with the preimmune rabbit serum. The specificity of the antibodies was confirmed by depletion experiments, using antisera preincubated with the peptide used for immunization (Fig. 3A). Specific signals were also observed by fluorescence microscopy in the cytoplasm of differentiated U937 cells (Fig. 3B) and by analytical flow cytometry of

RTP/Drg1/Cap43	tct	ctg	gat	ggc	acc	cgc	agc	cgc	tcc	cac	acc	agc	gag	ggc	acc
	S	L	D	G	T	R	S	R	S	H	T	S	E	G	T
Ndr1	-	E	-	-	-	-	-	-	-	-	-	-	-	-	P
TDD5	P	H	-	P	V	W	L	Q	C	-	I	L	-	-	-

cga	agc	cgc	tcc	cac	acc	agc	gag	ggc	acc	cgc	agc	cgc	tcg	cac	acc	agc	gag	ggg
R	S	R	S	H	T	S	E	G	T	R	S	R	S	H	T	S	E	G
-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	D
-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	D

Fig. 2. Nucleotide and amino acid sequence alignment near the C-terminus part of *RTP/Drg1/Cap43* human gene, and mouse *TDD5* and *Ndr1* genes, showing the highly hydrophilic region formed by ten amino acid units tandemly repeated three times (gray boxes). The 20-mer synthetic peptide, which was used as immunogen, is underlined. Amino acids that are identical to those in the Drg1 sequence, are shown as dashes.

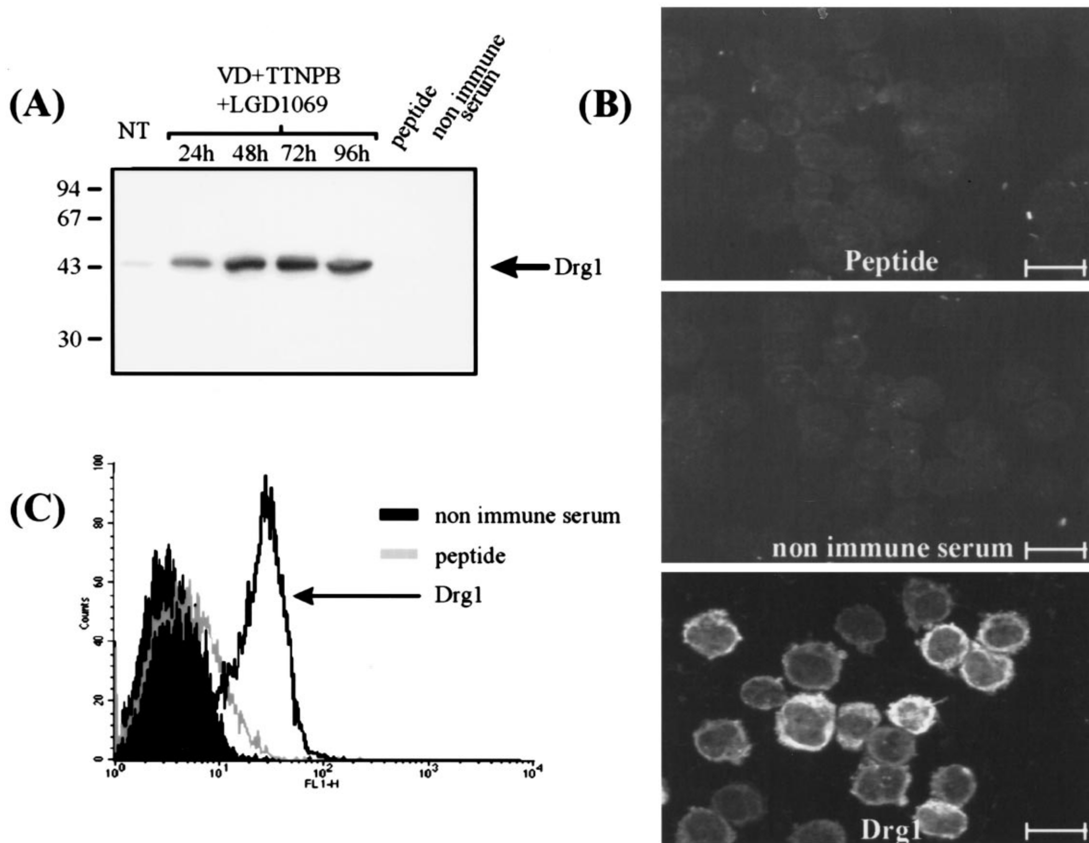

Fig. 3. Characterization of Drg1 in U973 cells. U973 cells were cultured at different times in the absence (NT) or presence of VD (100 nM), TTNPB (100 nM) and LGD1069 (1 µM). (A) Time course of Drg1 induction in U973 cells. Western blot was revealed by ECL with anti-Drg1 antibodies. The specificity of the band was checked by depletion experiment using polyclonal antibodies against RTP/Drg1/Ndr1 preincubated with the peptide used for immunization and using non-immune rabbit serum. Marker size is indicated in kilodaltons. (B) Confocal microscopy analysis of Drg1 expression. U937 cells were cultured for 48 h with differentiation inducers as in A. Fixed cells were stained with anti-Drg1 antibodies and revealed by rhodamine conjugated goat anti-rabbit F(ab)′2. Controls were achieved using antibodies preincubated with the peptide used for immunization and using non-immune rabbit serum. (C) Expression of Drg1 analyzed by flow cytometry. U937 cells were cultured for 48 h with differentiation inducers and stained by indirect immunofluorescence with anti-Drg1 antibodies (black line). Controls were achieved using antibodies preincubated with the peptide used for immunization (gray line) and using non-immune rabbit IgG (black line, filled histogram).

permeabilized cells (Fig. 3C). Increased expression of the Drg1 protein was observed in U937 cells treated with VD and the synthetic retinoids TTNPB and LGD1069 (Fig. 3A), under conditions where an increased expression of Drg1 mRNA was originally detected by differential display and further confirmed by Northern blot analysis (Fig. 1C).

3.3. *Expression of the RTP/Drg1/Ndr1 protein during cell growth arrest and differentiation*

We first investigated the effects of retinoids (RA or RAR synthetic agonists) on U937 cells and other cell types. The proliferation of U937 cells is inhibited to some extent and cells express some monocyte-macrophage functions following treatments with RA. Retinoids also inhibit the estradiol-induced proliferation of human MCF-7 mammary carcinoma cells [11,12]. In the case of C2 myoblasts, retinoids are efficient inducers of both growth arrest and differentiation [8]. In all these cases, we found an increased expression of a 43 kDa protein revealed by anti-Drg1 antibodies (Figs. 3–5).

Studies with other inducers or cell culture conditions revealed more complex patterns. VD, an inducer of monocytic differentiation with some effects on U937 cell growth inhibition, was able to induce the expression of Drg1. TGF-β1, which strongly potentiates the effects of VD on U937 cell growth inhibition and monocytic differentiation [13], had no significant effect on Drg1 expression either alone or in combination with VD (Fig. 4A). Conversely, retinoids, which also synergize with VD to induce cell growth inhibition and differentiation of U937 cells [1], co-operated with VD to stimulate Drg1 expression (Fig. 4A). Phorbol myristate acetate (PMA), which exerts a potent effect on growth arrest and monocyte-macrophage differentiation of U937 cells, was found to up-regulate the protein (Fig. 4A). A positive effect of PMA was also detected in mouse macrophagic J774 cells (Fig. 4B).

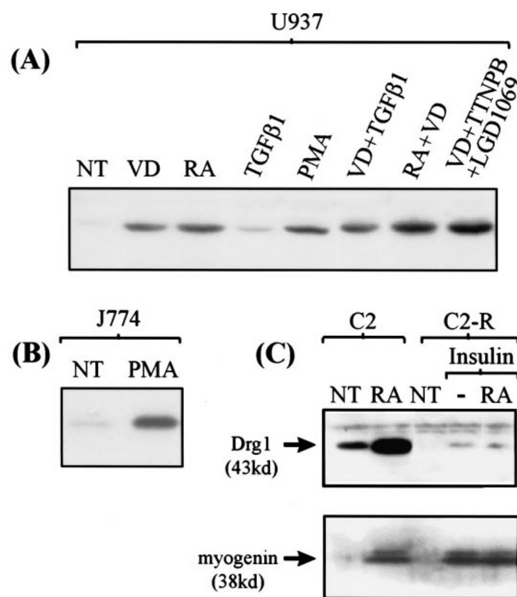

Fig. 4. Immunologic detection of RTP/Drg1/Ndr1 protein during cell differentiation. Cells were cultured for 48 h (except for C2 and C2-R, cultured for 72 h) with different combinations of VD, RA, TTNPB (100 nM each), LGD1069 (1 μM), insulin (1 μM) and PMA (100 ng/ml). Western blots were revealed by ECL with anti-Drg1 antibodies. (C) Myogenin was immunolabeled with mouse anti-myogenin antiserum.

Myogenic C2-R cells have been characterized as C2 subclones resistant to growth inhibition and differentiation by RA [8]. These cells can be differentiated by incubation in a poorly mitogenic medium (1% FCS) containing insulin (1 μM). After 3 days of incubation, they express myogenin, a muscle specific marker of terminal differentiation, at a level similar to that observed in the parental RA-differentiated C2 cells (Fig. 4C). We observed that proliferating C2-R cells did not express the RTP/Drg1/Ndr1 protein. Upon differentiation in insulin-containing medium, they expressed a low level of RTP/Drg1/Ndr1 but we found that the presence of RA had no effect on this expression. The level of RTP/Drg1/Ndr1 remained very low, contrasting with the high level observed in the parental cell line (Fig. 4C).

To get a further insight into the effects of lipophilic agents on RTP/Drg1/Ndr1, we studied its expression in human mammary carcinoma MCF-7 cells. The growth of MCF-7 is estrogen-dependent. Cells cease to proliferate and accumulate in the G_1 phase of the cell cycle when suppressing estrogen stimulation [11,12]. We found that the basal level of RTP/Drg1/Ndr1 protein, observed in proliferating cells cultured in the presence of 1 μM estradiol (E_2), was not increased when removing E_2 from the culture medium (Fig. 5A). In E_2-stimulated cells, the RAR-specific agonist TTNPB (10 nM) or the pure anti-estrogen ICI 182,780 (10 nM) failed to increase the protein level. At the same concentration, but in the absence of E_2, both agents stimulated its expres-

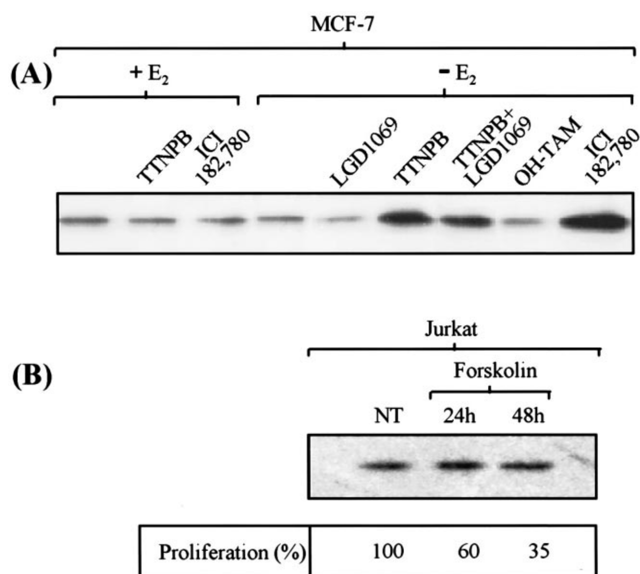

Fig. 5. Immunologic detection of RTP/Drg1/Ndr1 protein during cell growth arrest. MCF-7 cells were cultured for 48 h and Jurkat cells during different times, with different combinations of (A) E_2 (1 μM), TTNPB (100 nM), LGD1069 (30 nM), ICI 182,780 (10 nM), OH-TAM (10 nM) or (B) forskolin (100 mM). Western blots of cells during cell growth arrest were labeled with anti-Drg1 antibodies and revealed by ECL. In B viable cells (> 95%) were counted after staining with trypan blue.

sion (Fig. 5A). In cell cultures run in parallel, in the absence of E_2, the RXR-specific agonist LGD1069 and the partial anti-estrogen 4-hydroxytamoxifen (OH-TAM) had no significant effect on RTP/Drg1/Ndr1 protein expression and we did not detect co-operative effects between the RAR and RXR agonists (Fig. 5A).

Extending our investigations to other cell lines, we detected a basal expression of RTP/Drg1/Ndr1 in proliferating human leukemic Jurkat T-cells (Fig. 5B). We used forskolin (100 μM) ([6] and references therein) to inhibit Jurkat cell growth (respectively 40% inhibition after 24 h and 65% after 48 h) without detectable apoptosis, as assessed by the absence of annexin V at the cell surface (data not shown). This cell growth inhibition was not associated with an increased expression of the RTP/Drg1/Ndr1 protein (Fig. 5B).

4. Discussion

The emergence of new technologies for studying simultaneously the expression of large numbers of genes, together with the determination of a growing number of partial cDNA sequences (expressed sequence tags, EST), now allows to identify numerous novel genes in mouse and human species. *RTP*, [4], *Drg1* [3] and *Cap43* [5] refer to genes the products of which were independently found by mRNA differential display in various human cells and tissues. A cDNA clone (rit42) identical to RTP was identified using a differential screening technique, on the basis of its reduced expression in tumor cells [14]. In the present work, the inducible expression of *Drg1* was again observed in a mRNA differential display experiment. The predicted human protein sequences differ by only one amino acid and the minor differences between the nucleotide sequences do not allow to ascertain the existence of more than one gene in the human genome. In fact, the differences between human sequences might reflect allelic variations. A murine homologous gene, *Ndr1*, has been identified [15]. The mouse TDD5 predicted protein [16] differs significantly from other proteins in the C-terminal region and might thus represent another member of this family. In our work, the complete cDNA sequence was determined only in the case of U937 cells, indicating the product of a gene identical to *Drg1*. We thus used this term to name the protein revealed in this cell line and we tentatively used the generic term RTP/Drg1/Ndr1 to name the protein revealed in other cell types. A more accurate nomenclature awaits further progress in the characterization of this novel gene and protein family.

Until now, it has not been possible to find significant homologies between the predicted RTP/Drg1/Ndr1 proteins and any protein with a known function. A first step in understanding its biological role is to determine when and where this novel gene is expressed. The UniGene collection (http://www.ncbi.nlm.nih.gov/UniGene/) provides a non-redundant set of clusters, each one containing sequences that represent a unique gene or highly related genes. A recent UniGene release (No. 55, 8 October 1998) indicates that the cluster defined by *RTP*, *Drg1* and *Cap 43* (Hs 75789) also contains the sequences of 184 ESTs representing cDNA fragments and thus mRNAs originating from a wide variety of human tissues. The cluster containing the mouse *Ndr1* and *TDD5* sequences (Mm 4063) contains 70 ESTs se-

quences representing mRNAs extracted from brain, embryo, kidney, macrophage, mammary gland, muscle, skin, thymus and uterus. These data show that, at least at the transcriptional level, *RTP/Drg1/Ndr1* expression is not tissue-specific.

In the present work, we prepared antibodies to study *RTP/Drg1/Ndr1* expression at the protein level. We raised these antibodies against a repeated motif, which is unique to the putative proteins. RTP and Cap 43 have the same predicted peptide sequence and can be distinguished only by a few differences in the 3′-untranslated region of their mRNAs. Drg1 differs from the four other sequences by one amino acid (threonine instead of isoleucine) because of a T-to-C transition but this difference is not localized in the chosen epitope. The TDD5 predicted sequence contains two ten amino acid repeats which match with the synthetic peptidic antigen. Ndr1 has the three characteristic tandem repeats, and the two amino acid changes by which they differ from the human sequence are substitutions to similar residues. Therefore, we expected the antibodies to recognize all the predicted proteins. In all cell extracts, either human or murine, where a positive reaction was registered, a unique band was observed in Western blot with the expected apparent molecular mass (43 kDa). In differentiated U937 cells, cell labeling showed strong cytoplasmic staining, as observed in differentiated colon carcinoma cells [3]. The diversity of cell types in which we observed a positive band in Western blot experiments confirmed, at the protein level, the lack of tissue specificity and widespread occurrence of *RTP/Drg1/Ndr1* gene products previously seen by others at the mRNA level (Table 1). These studies also confirmed that the level of expression of *RTP/Drg1/Ndr1* is highly variable depending on cell type and culture conditions, thus accounting for the reiterated discovery of its transcription products by several groups of investigators using differential screening techniques. The emerging picture is that *RTP/Drg1/Ndr1* is a widely expressed gene, coding for a cytoplasmic protein, which may be translocated in the nucleus [3,14], which seems to be down-regulated in tumor cells and up-regulated in cells which differentiate and cease to proliferate. This regulation is complex and different cases must be considered. (i) The protein may be present but not up-regulated upon cell growth arrest. Removing estradiol from the culture medium of MCF-7 cells or treating Jurkat T-cells with forskolin did not increase the basal expression observed in proliferating cells. A lack of *Drg1* induction in cells which cease to proliferate was also reported in A431 epidermoid carcinoma cells [3]. These data suggest that the signals triggering *RTP/Drg1/Ndr1* induction may not depend merely on cell cycle arrest. (ii) Inductions of *RTP*, *Drg1*, *Cap43* and *rit42* have been observed upon treatments involving some forms of cellular stress (respectively exposure to reducing agents or tunicamycin, glucose starvation, nickel compounds, DNA damaging agents). In HUVEC, RTP mRNA was induced in parallel with the mRNA of GRP78/BiP, a molecular chaperone up-regulated by the same toxic agents [4]. More recently, up-regulation of *RTP/rit42* was found closely associated with p53 expression [14]. In normal colon, *Drg1* is expressed at the terminal stage of differentiation, just preceding apoptosis and shedding of cells in the colon lumen [3]. Induction of *RTP/*

Table 1
Cell types and treatments in which *RTP/Drg1/Ndr1* gene expression was found to be up-regulated

Cell type	RTP/Drg1/Ndr1 expression inducers
Endothelial cells [4,20] (HUVEC)	Homocysteine, 2β-mercaptoethanol, Tunicamycin, lysophosphatidylcholine
Colon carcinoma cell [3] (HT29-D4, Caco-2)	Decreased glucose concentration
Pulmonary epithelial cells [5] (A549, HTE, Calu-1)	$NiCl_2$, Ni_3S_2
Lung fibroblasts [5] (WL-38)	$NiCl_2$, Ni_3S_2
Osteosarcoma [5] (HOS)	$NiCl_2$, Ni_3S_2
Null-p53 cell lines [14]	p53, DNA-damaging agents
Myelomonocytic cells (U937)	Retinoids, vitamin D, PMA
Macrophagic cells (J774) (mouse cells)	PMA
Mammary carcinoma cells (MCF-7)	Anti-estrogen ICI 182,780, RAR agonist TTNPB
Myoblast cells RA sensitive (C2) (mouse cells)	Retinoic acid

Drg1/Ndr1 might thus be linked to cellular stress, terminal differentiation leading to senescence and cessation of proliferation preceding apoptosis. (iii) The wide cellular distribution of *RTP/Drg1/Ndr1* transcripts suggests that their expression depends on diverse stimuli. Here, we report the induction of *RTP/Drg1/Ndr1* by VD and retinoids, agents known to play biological roles in the development of embryos and in the control of tissue homeostasis in the adult. Van Belzen et al. also described a retinoic acid induction of Drg1 expression [17]. The pleiotropic effects of retinoids, VD and other lipophilic signals on cell growth and differentiation are mediated by nuclear receptors (RARs and RXRs and VDR) which belong to the superfamily of ligand inducible transcription factors. A characteristic feature of this family of receptors is that they act as heterodimers, in which RXRs play a central role since they form heterodimers with the other nuclear receptors, including RARs and the VD receptor [18,19]. Depending on the cellular context, the RXR receptor may or may not be itself ligand activatable by specific retinoids. These interactions offer multiple possibilities of cross-talk between lipophilic endocrine signals, so that each cell type exhibits its own pattern of reactivities. In the present work, we have studied the expression of the protein in three well-established experimental cell models. In U937 cells, where the differentiation induced by VD is potentiated by RXR and RAR-specific ligands [1], the extent of induction of Drg1 was in agreement with the potency of combinations of differentiation inducers. Drg1 was induced both by VD and RA alone, but the highest protein level was found in cells treated by a combination of VD, RAR and RXR agonists previously shown to give optimal effects on differentiation, leading to cells which are not apoptotic at this stage and which express multiple macrophage-specific functions ([1] and references therein). The strong and early (4 h) induction of mRNA, which originally justified the selection of *Drg1* in our mRNA differential display experiment, shows that a major regulation occurs at the transcriptional level. An induction in response to RAR-, but not RXR-specific retinoids was also observed in MCF-7 cells. In this case, we found that *RTP/Drg1/Ndr1* expression was also controlled by molecules having a pure anti-estrogenic effect (ICI 182,780), but not by other anti-estrogens (OH-TAM), suggesting a complex network of interactions at the level of ligand-activatable nuclear transcription factors. The sensitivity to RA was also illustrated by the very different behavior of myogenic C2 and C2-R cells, the conspicuous expression of the protein in RA-sensitive cells contrasting with its very low expression in RA-resistant cells brought to the same stage of muscle differentiation. It will thus be interesting to investigate the structure of the Drg1 promoter looking for responsive elements characteristic of the VDR/RAR/RXR family. At present, nothing is known on the biological role of *RTP/Drg1/Ndr1*. However, it seems clear that it is associated with, and probably plays itself a role [14] in a cellular mechanism involving the loss of proliferative potential.

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