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Nuclear Translocation of YB-1 Protein Induced by Ultraviolet Light and the Investigational Gallium Drug GaQ3 in Melanoma Cells

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1. Introduction

The Y-box factors are a family of proteins that have been structurally and functionally conserved throughout evolution (Wolffe et al., 1992). They are transcription factors that are expressed in a wide range of cell types from bacteria to human cells. They are called Y-box proteins after the specificity originally shown for human YB-1 in binding the Y-box sequence CTGATTGGCCAA present in the promoter region of major histocompatibility complex class II genes (Didier et al., 1988). Y-box proteins have emerged as key players in cellular metabolism. These proteins are capable of binding one or more types of nucleic acids, single- or double-stranded DNA or RNA. The negative or positive modulation of transcription by YB-1 causes diverse biological effects on a wide array of genes, modification of chromatin (Ashizuka et al., 2002), translational masking of mRNA (Evdokimova et al., 2006), cooperation in eukaryotic redox signaling pathways (Swamynathan et al., 2002), RNA chaperoning (Matsumoto et al., 2005) and stress response regulation (Swamynathan et al., 1998). The nuclear functions of YB-1 also include a role in DNA replication (En-Nia et al., 2005) and repair (Marenstein et al., 2001) as well as mRNA transport into the cytoplasm (Soop et al., 2003) (Figure 1).

YB-1 protein is a member of the DNA-binding protein family. It binds to the Y-box, an inverted CCAAT box, in the promoter region of many oncogenes and regulates their activity, for example the human multidrug resistance 1 gene, which encodes P-glycoprotein (P-gp) (Kuwano et al., 2004; Gluz et al., 2009), the cell division cycle 6 gene, which promotes cell cycle progression (Basaki et al., 2010), and the membrane type I-matrix metalloproteinase, which facilitates tumor invasion and metastasis (Lovett et al., 2010).

P-glycoprotein was first identified by virtue of its overexpression in multidrug resistant (MDR) tumor cells, where it mediates the energy-dependent efflux of a variety of chemotherapeutic agents (Trambas et al., 1997). The human class I P-glycoprotein is known to transport phospholipids, cholesterol, calcium channel blockers, immunosuppressants, peptides, steroids and xenobiotics (Bauer et al., 2005). More recent studies suggest that

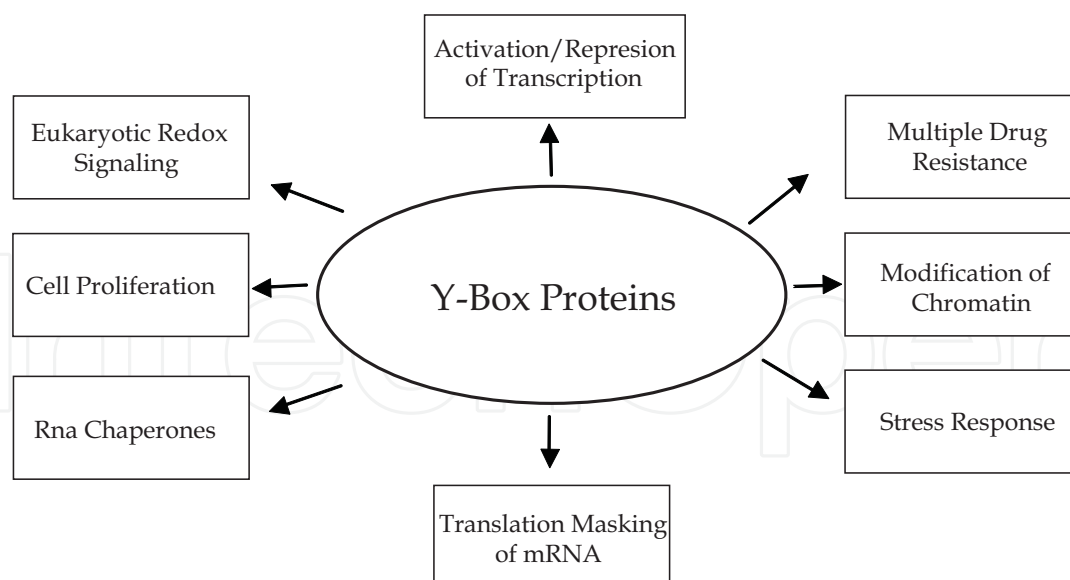


Fig. 1. Involvement of Y-box proteins in various cellular processes

upregulation of P-gp genes in tumor cells can prevent apoptosis and enhances the ability to resist chemotherapeutics (Park et al., 2006) and a wide range of apoptotic inducers, including serum starvation, fas ligand, UV irradiation, tumor necrosis factor and other stress inducer, including heat shock and chemotherapeutic agents (Hu et al., 2000). Gómez-Martínez has reported that the proximal promoter of MDR 1 contains several regulatory regions, including an inverted CCAAT box at -82 to -73 and a GC element at -56 to -42, both of which have been shown to be necessary for constitutive promoter activity in some cell types (Gómez-Martínez et al., 2007). Various environmental stimuli increase the activity of the MDR 1 promoter in response to all stimulation cascades that are dependent on the inverted CCAAT box (Kathleen & David, 1998). Interaction of the nuclear factor MDR-NF1 with this promoter region is increased when cells are exposed to UV or anticancer drugs (Ohga et al., 1996). To understand the molecular basis of MDR 1 promoter activity and the stress-dependent induction, the cDNA for MDR-NF1 has been cloned, revealing that the amino acid sequence encoded by the cloned cDNA is identical to that of YB-1 (Ohga et al., 1996). YB-1 was found to be expressed at much higher levels in all cisplatin-resistant cell lines than in the respective drug-sensitive parental counterparts. Two transfectants with a YB-1 antisense construct showed increased sensitivity to cisplatin, mitomycin C and UV irradiation, but not to vincristine, doxorubicin, camptothecin or etoposide, suggesting that cells with downregulated YB-1 gene expression are more sensitive to agents that induce cross-linking of DNA (Ohga et al., 1996). Nuclear localization and an upregulation of YB-1 expression are closely associated with intrinsic MDR 1 gene expression in primary human breast cancers (Bargou et al., 1997; Spitkovsky et al., 1992). YB-1 protein is localized mainly in the cytoplasm, but accumulates in the nucleus by UV exposure, hyperthermia (Stein et al., 2001), phosphorylation (Davies et al., 2011), or association with p53 (Zhang et al., 2003). This translocation may be induced by a protein kinase C-mediated signal transduction pathway, and the C-terminal region of YB-1 might be important for cytoplasmic retention of YB-1 (Koike et al., 1997). Similarly, exposure to chemotherapeutic agents stimulates accumulation in the nucleus (Valiahdi et al., 2006).

In malignant melanoma, YB-1 plays a key role for proliferation, survival and invasion and increases chemoresistance. The high expression of YB-1 in aggressive types of cancer and the

evidence for a role in cell proliferation suggests a potential as a therapeutic target. YB-1 also associates with key tumor factors such as MMP-2, bcl-2, cyclinD1 and p16INK4A (Schitteck et al., 2007), p53 (Okamoto et al., 2000), AP-1 (Lasham et al., 2003), Smad 3 and p300 (Higashi et al., 2003) and directly as well as indirectly regulates gene expression. An increased YB-1 expression of melanoma cells in comparison to benign melanocytes was shown in vitro and in vivo (Schitteck et al., 2007). YB-1 is translocated into the nucleus in invasive and metastatic melanoma cells, and a downregulation of YB-1 by shRNA reduces the rate of proliferation and increases the rate of apoptotic cell death (Schitteck et al., 2007). Translocation of the protein into the nucleus leads to its association with the family of serine/threonine kinases that inhibit apoptosis, stimulate angiogenesis, and promote tumor formation. It has also been suggested that Janus kinase and casein kinase II phosphorylate YB-1 and induce translocation of this protein into the nucleus (Sutherland et al., 2005).

Gallium has long been studied for its suitability both for imaging and therapy of malignant tumors including melanoma among others, with the most prominent effects in malignant lymphoma (Jakupec & Keppler, 2004). Though never established in clinical routine, attempts to utilize gallium-67 scintigraphy for the detection and surveillance of occult metastatic disease in melanoma patients showed that these tumors can regularly be visualized by gallium tracers, suggesting a certain affinity (Kirkwood et al., 1982). To investigate the therapeutic effect of gallium nitrate in advanced malignant melanoma, a clinical study was performed in which gallium nitrate (250-300 mg/m²) was administered daily to 31 patients for 7 consecutive days. The compound was well tolerated, but only one of 31 evaluable patients experienced a partial remission (Casper et al., 1985). In a prior phase I study, 3 of 19 patients with advanced melanoma treated with gallium nitrate had experienced disease stabilization (Bedikian et al., 1978).

In order to improve the oral bioavailability and the pharmacological activity of gallium, a gallium complex has been developed by our institute (GaQ3). GaQ3 is an investigational anticancer drug in early clinical development, suitable for oral administration and well tolerable in toxicological studies (Collery et al., 1996). We demonstrated strong antiproliferative effects of GaQ3 in metastatic melanoma cell lines and in primary cultures of freshly explanted melanoma samples in comparison to established drugs (Valiahdi et al., 2009).

2. Experimental part

2.1 Drugs

Tris(8-quinolinolato)gallium (III) (GaQ3) was synthesized and isolated as a solid in high purity at the Institute of Inorganic Chemistry, University of Vienna, Austria, according to a previously established procedure (Collery et al., 1996). Gallium nitrate was purchased from Sigma-Aldrich as a hydrate of the composition Ga(NO₃)₃·5.8H₂O, as determined by thermogravimetric analysis. GaQ3 was applied from fresh stock solutions in dimethylsulfoxide (DMSO) and diluted in media or buffer as appropriate to a maximum of 0.3% DMSO.

2.2 Cell lines and culture conditions

The human melanoma cell lines 518A2, 607B, A375, MEL-JUSO and SK-MEL-28 were kindly provided by Dr. Rodrig Marculescu (Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Vienna, Austria) and chicken embryo fibroblasts (Cef32)

by Prof. Dr. Wolfgang Schneider (Max F. Perutz Laboratories; Medical University of Vienna; Austria) , respectively. Cells were grown in 75 cm² culture flasks (Iwaki/ Asahi Technoglass, Gyouda, Japan) as adherent monolayer cultures in complete culture medium, i.e. minimal essential medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 4 mM L-glutamine and 1% non-essential amino acids (100×) (all purchased from Sigma–Aldrich, Vienna, Austria). Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO₂.

2.3 Cytotoxicity assay

Cytotoxicity was determined by a colorimetric microculture assay (MTT assay). For this purpose, 518A2, 607B, A375, MEL-JUSO and SK-MEL-28 cells were harvested from culture flasks by trypsinization and seeded into 96-well microculture plates (Iwaki/Asahi Technoglass) in densities of 1.5–3.5 × 10⁴ viable cells/well, depending on the cell line. After a 24 h preincubation, cells were exposed to dilutions of the test compounds in 200 µL/well complete culture medium for 96 h. At the end of exposure, drug solutions were replaced by 100 µL/well RPMI 1640 medium (supplemented with 10% heat-inactivated fetal bovine serum and 4 mM L-glutamine) plus 20 µL/well MTT solution (5 mg/mL) in phosphate-buffered saline (PBS) . After incubation for 4 h, medium was removed and the formazan product formed by viable cells was dissolved in DMSO (150 µL/well). Optical densities at 550 nm were measured with a microplate reader (Tecan Spectra Classic). The quantity of viable cells was expressed in terms of T/C values by comparison to untreated controls, and 50% inhibitory concentrations (IC₅₀) were calculated from concentration-effect curves by interpolation. Evaluation is based on means from at least three independent experiments, each comprising six replicates per concentration level.

2.4 Construction of plasmids expressing a GFP-YB-1 fusion protein

Because of the high levels of endogenous YB-1 in the cells, it was useful to use tagged YB-1 constructs for the study of YB-1. In this study, we developed a tagged chk-YB-1 by cloning cDNA of YB-1 into the ApaI site of the pEGFP-C1 plasmid. To investigate role of extreme C-terminus of YB-1 on translocation into nucleus, the full-length YB-1 gene was deleted at the C-terminus to create the YB-1Δ27 product (Figure 2).

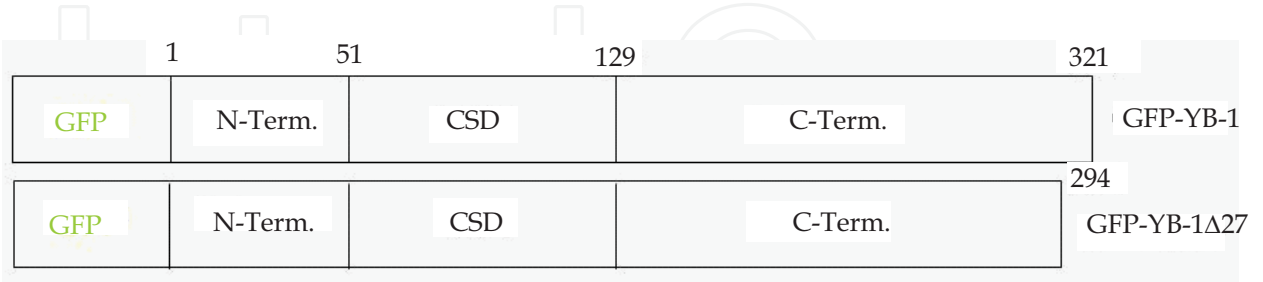


Fig. 2. Schematic representation of GFP-YB-1 and the truncated construct GFP-YB-1Δ27. The numbers represent the position of amino acids at the border between the respective domains. CSD: cold shock domain; GFP: green fluorescent protein

2.5 Transient transfection

Cef32 cells (chicken embryo fibroblasts) were transfected with a GFP-chk-YB-1, GFP-chk-YB-1Δ27 or GFP only construct. SK-MEL-28 cells were transfected with a pCMV-YB-1-GFP

construct (kindly provided by Dr. K. Higashi, Kyoto University, Kyoto, Japan). In a six-well plate or 35-mm tissue culture plate, about $1-2 \times 10^5$ cells were seeded in 2 ml of MEM supplemented with 10% fetal calf serum and grown for 24 h to a confluence of 40-60%. The cells were washed once with 2 ml of serum-free medium, and for each transfection 0.8 ml serum-free medium were added to each tube containing 200 μ l jetPEI reagent-DNA complex (Qbiogene, Inc). After washing, cells were overlaid with a 1 ml mixture of jetPEI reagent-DNA complex and serum-free medium and incubated for 5-24 h at 37 °C. Then the DNA-containing medium was replaced with 2 ml of growth medium containing serum and incubated for 48-72 h. In order to assure successful transfection, the expression of the reporter gene encoding for green fluorescence protein (GFP) was visualized directly by observation of green fluorescence under the fluorescence microscope or indirectly by an anti-GFP antibody (Western blotting).

2.6 Nuclear and cytoplasmic cell extracts

To obtain total cell extracts, Cef32 cells were washed twice in PBS, scraped into PBS, pelleted, and resuspended in lysis buffer. Cells were washed with PBS, resuspended in 200 μ l ice-cold 40% TKM buffer (50 mM Tris-HCl pH 7.4, 25 mM KCl, 5 mM MgCl₂, 0.2 mM EDTA) containing protease inhibitor (1 M dithiothreito (DTT), 10 mM vanadate, 1 mg/ml leupeptin, 3 mg/ml aprotinin and 100 mM phenylmethylsulfonylfluorid (PMSF)) and incubated on ice for 10 min. Then, the cells were disrupted by 25 strokes in a dounce homogenizer using a tight-fitting pestle. The homogenate was immediately diluted with the threefold volume of TKM buffer and centrifuged at 1000xg for 10 min at 4 °C. The low-speed supernatant was used as the cytoplasmic fraction. The low-speed pellet was resuspended in 400 μ l TKM buffer, laid on 400 μ l 60% sucrose in TKM and centrifuged at 100.000xg for 60 min at 4 °C. The sucrose was aspirated and the pellet (nuclear fraction) resuspended in 800 μ l TKM buffer.

2.7 Western blotting

Western blots of both nuclear extracts and cytosolic fractions of untreated and UV-exposed Cef32 cells were assayed by immunoblotting using anti-chk-YB-1C (1:5000 dilution, Sigma) and primary antibodies against GFP (1:1000) (mouse IgG monoclonal antibody, clones 7.1 and 13.1, Roche). For this purpose, Cef32 cells and Cef32 cells transiently expressing EGFP-chk-YB-1 were treated with UV light (50 J/m²), and whole cell lysates or fractionated cell lysates were diluted in 2x sample buffer (125 mM Tris pH 6.8, 4% sodium dodecyl sulfate (SDS), 10% glycerol, 0.006% bromophenol blue, 1.8% beta-mercaptoethanol). Denatured cellular extracts were resolved by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis), transferred onto nitrocellulose membranes (Amersham Hybond ECL, GE Healthcare, Little Chalfont, UK), blocked in fetal calf serum, and incubated with appropriate antibodies. Secondary anti-mouse antibody was used in dilutions of 1:10000. For visualization of the proteins, Western blotting luminal reagent (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used according to the instructions of the manufacturer, and X-ray film was exposed to the blots.

2.8 UV irradiation

Cef32 cells were grown in MEM medium to 80% confluence. The medium was removed and the cells were washed with PBS (37 °C). After treatment of the cells with UV light at 50 J/m²

(UV Stratalinker 2400), the removed medium was added again to the cells and cells were incubated for 1 hour at 37 °C. The effect of UV irradiation was investigated either by using fluorescence microscopy or Western blotting.

3. Results

Cytotoxicity in melanoma cell lines

The IC₅₀ values obtained with GaQ3 in five human melanoma cell lines are in a relatively narrow range of very low micromolar concentrations (0.8–2.5 μM), whereas cytotoxicity of the former investigational anticancer drug gallium nitrate is much lower, as reflected by IC₅₀ values between 20 and > 200 μM in the same cell lines (Table 1, Figure 3). Thus, GaQ3 shows a 23- to > 80-fold higher potency than gallium nitrate, based on the comparison of IC₅₀ values. The high intrinsic gallium nitrate resistance of the cell line SK-MEL-28 does not affect sensitivity to GaQ3 in equal measure.

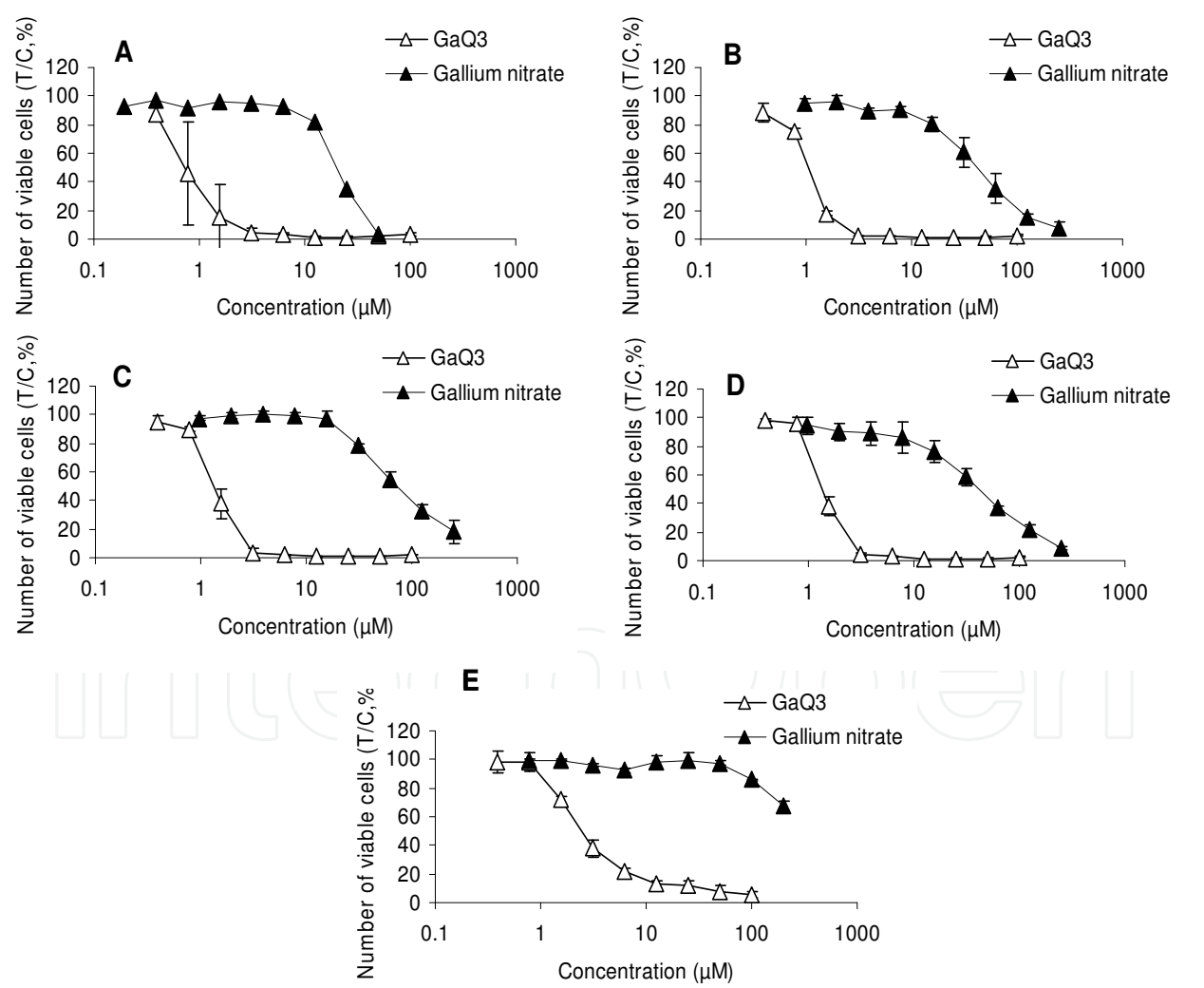


Fig. 3. Comparison of concentration-effect curves of GaQ3(–△–) and gallium nitrate (–▲–) in the human melanoma cell lines 518A2 (A), 607B (B), A375 (C), MEL-JUSO (D) and SK-MEL-28 cells (E), obtained by the MTT assay (96 h exposure)

Compound	IC ₅₀ (μM) ^a				
	SK-MEL-28	607B	A375	MEL-JUSO	518A2
GaQ3	2.45 ± 0.15	1.35 ± 0.09	1.30 ± 0.15	1.05 ± 0.10	0.855 ± 0.362
Gallium nitrate	> 200	39.9 ± 4.7	72.4 ± 8.9	42.7 ± 9.1	20.0 ± 1.0

^a 50% inhibitory concentrations in SK-MEL-28, 607B, A375, MEL-JUSO and 518A2 melanoma cells in the MTT assay, 96h exposure. Values are the mean ± standard deviation obtained from at least three independent experiments.

Table 1. Cytotoxicity of the new gallium complex (GaQ3) in comparison with gallium nitrate in five human melanoma cell lines

Immunostaining

Preliminary experiments showed that the chk-YB-1C antibody was unable to detect the fusion YB-1 protein, presumably due to steric hindrance. Therefore, exogenous YB-1 expression in GFP-YB-1 transfected Cef32 cells (in comparison with GFP-only-transfected cells) were indirectly detected by an anti-GFP antibody (Figure 4). The expression of the fusion protein GFP-YB-1 with a molecular weight of 77 kDa is clearly discernible in the immunoblots of total Cef32 cell extracts.

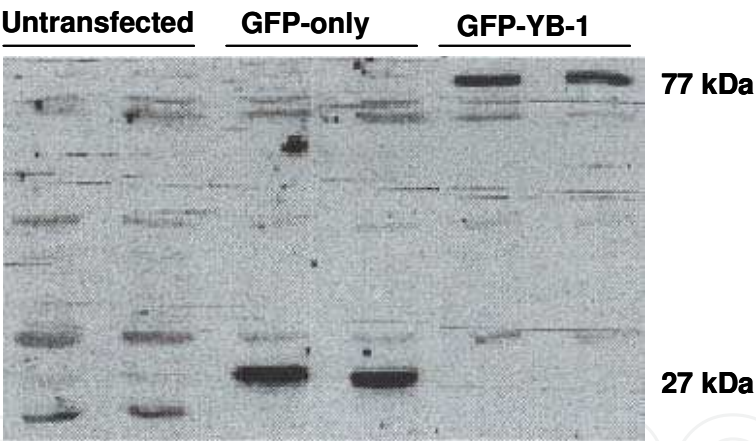


Fig. 4. Western blot of total cell extracts from Cef32 cells transiently transfected with GFP and GFP-YB-1, as well as from untransfected cells (negative control). Proteins were separated on a 10% polyacrylamide SDS gel and probed with anti-GFP antibody (1:5000 dilution)

The effect of UV irradiation on the localization of endogenous and exogenous chk-YB-1 in Cef32 cells was studied with an antibody directed against the extreme C-terminus of YB-1 (anti-chk-YB-1C), revealing that the endogenous protein in UV-exposed cells can be found in the nuclear fraction after only 1 hour incubation (Figure 5, middle blots). To indicate the exogenous YB-1 protein in Cef32 cells, we detected the presence of the GFP fusion protein in the nuclear fraction after UV irradiation by an anti-GFP polyclonal antibody, revealing the same nuclear translocation for the exogenous YB-1 protein upon UV exposure (Figure 5, upper blot).

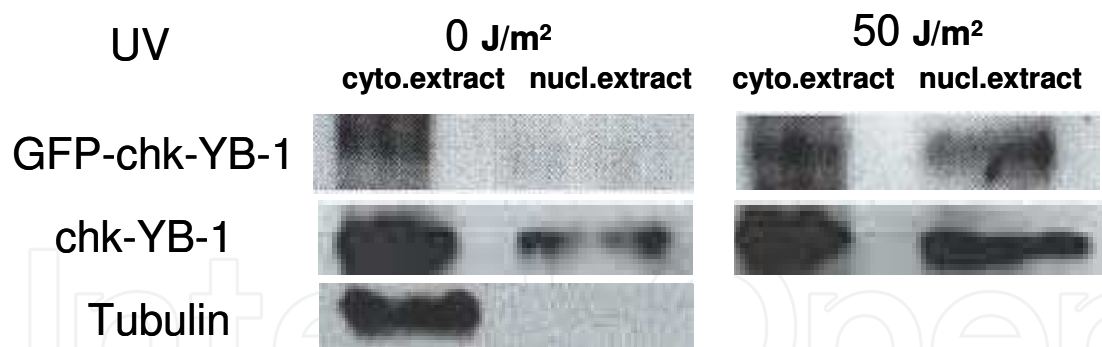


Fig. 5. Subcellular distribution of endogenous and exogenous YB-1 protein in Cef32 cells. Western blots of both nuclear extracts and cytosolic fractions of untreated and UV-exposed (50 J/m²) untransfected and transfected Cef32 cells were assayed by immunoblotting using anti-GFP antibody (upper blots) and anti-chk-YB-1C antibody (middle blots). In order to exclude contamination of the nuclear fraction with cytosolic proteins, extracts were immunoblotted with anti- α -tubulin (lower blot)

Fluorescence microscopy

The subcellular distribution of GFP-YB-1 and the effect of UV irradiation were additionally investigated by fluorescence microscopy of Cef32 cells transfected with the chk-YB-1-GFP expression construct, yielding further evidence for the nuclear accumulation of YB-1 upon UV irradiation, whereas larger amounts of YB-1 were detected in the cytosol, more at the perinuclear region than in the nucleus of untreated fibroblast cells (Figure 6).

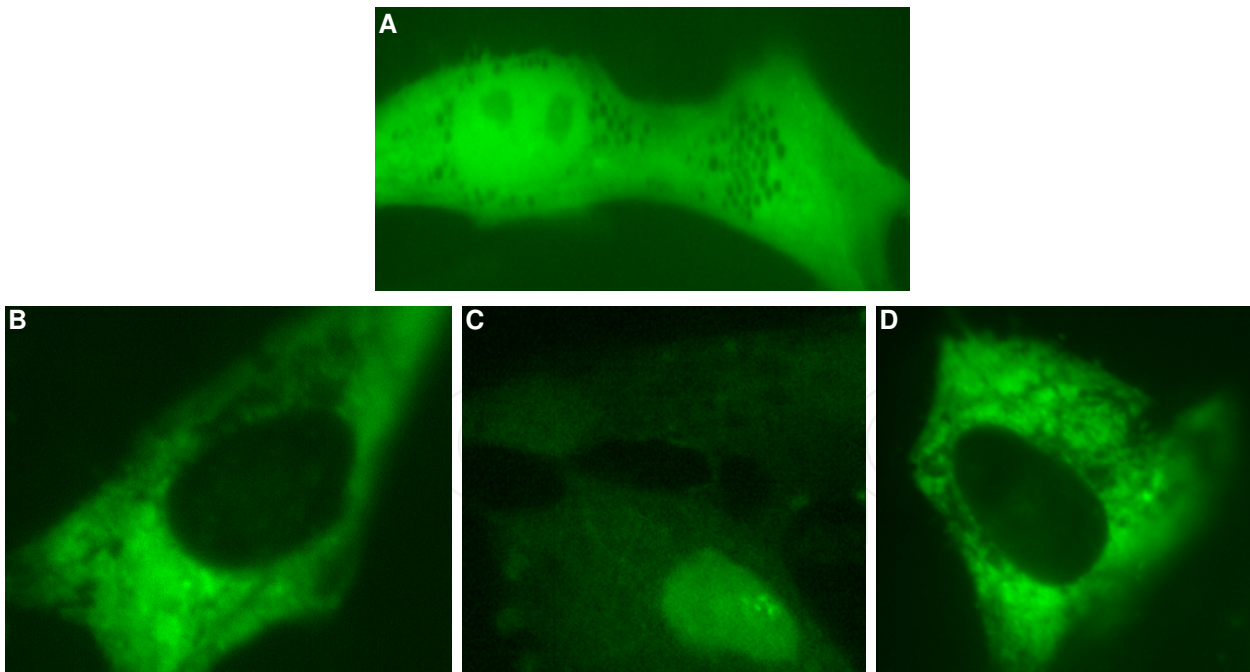


Fig. 6. Fluorescence microscopic images of Cef32 cells transfected with either pEGFP-C1 only (A) or a GFP-chk-YB-1 construct (B-D). While the fluorescence of GFP is rather evenly distributed in both cytosol and nucleus in the former cells (no difference of GFP expression in cytoplasm and nucleus) (A), untreated GFP-chk-YB-1 transfected cells show mainly cytosolic fluorescence, indicating the cytosolic expression of YB-1 (B, D). Translocation of this protein into the nucleus was observed 2 h after UV irradiation (50 J/m²) (C)

To investigate the effect of the deletion of the 27 C-terminal amino acids on intracellular localization of YB-1, GFP-YB-1(27 was transfected transiently into Cef32 cells and visualized by fluorescence microscopy after 48 hours incubation at 37 °C. GFP-YB-1 and GFP-YB-1(27 lacking the C-terminus (294-321) of YB-1 were located mainly in the cytosol, but the deletion construct was located more in perinuclear regions (Figure 7 B). In contrast to cells transfected with the complete GFP-YB-1 construct, UV irradiation did not result in recognizable nuclear translocation of fluorescence in those cells transfected with the deletion construct (Figure 7C), suggesting that the C-terminal sequence is essential for translocation.

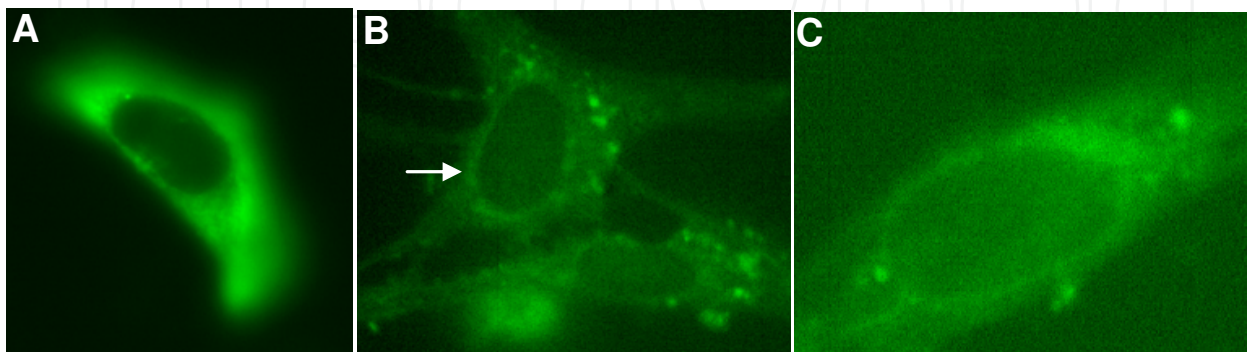


Fig. 7. Fluorescence microscopic images of Cef32 cells transiently transfected with GFP-YB-1 (A) or GFP-YB-1 Δ 27 (B, C). The deletion product GFP-YB-1 Δ 27 is mainly expressed in the cytosol, in particular in the perinuclear region, in untreated cells (B), and no pronounced translocation into the nucleus is discernible upon UV irradiation (50 J/m²) (C)

Furthermore, the human melanoma cell line SK-MEL-28 was transfected with a pCMV-hYB-1-GFP plasmid (encoding for human YB-1), and the localization of YB-1 in the cell was again visualized using the fluorescence of the reporter protein GFP. The same translocation of YB-1 into the nucleus as in UV-treated Cef32 cells was observed upon GaQ3 treatment in these transiently transfected SK-MEL-28 cells (Figure 8).

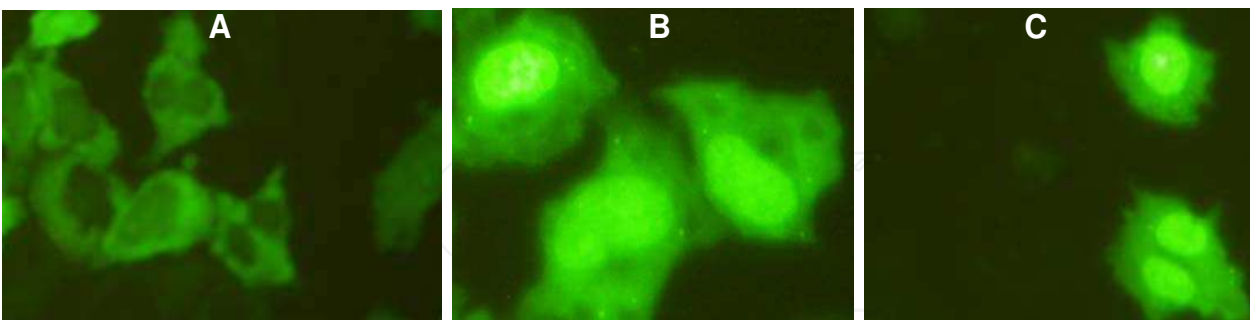


Fig. 8. Fluorescence microscopic images of SK-MEL-28 cells transiently transfected with pCMV-YB-1-GFP construct. Untreated cells show mainly a cytosolic expression of YB-1 (A), whereas translocation into the nucleus is visible after treatment with 5 μ M GaQ3 for 24 h (B,C)

4. Discussion

The tumor-inhibiting compound GaQ3 has strong antiproliferative activity in human melanoma cells, in particular in the Human Tumor Cloning Assay (HTCA) in primary melanoma explants. The effects induced by GaQ3 in melanoma cell lines involve S phase

arrest, caspase activation and poly-(ADP-ribose) polymerase cleavage, indicating induction of apoptosis, whereas no direct interactions with DNA could be observed (Valiahdi et al., 2009). The improved pharmacological properties of GaQ3 as compared to earlier gallium-based agents and in vitro data presented here warrant further studies of this compound in malignant melanoma. The mechanism of action of GaQ3 is far from understood, but seems to deviate from that of gallium nitrate and does not involve DNA interactions such as cross-linking or strand breakage (Valiahdi et al., 2009). Nevertheless, we could demonstrate that cellular responses to this investigational anticancer drug involve the Y-box protein YB-1.

Y-box proteins play multiple roles in the cell, serving as transcription factors, as a part of mRNA particles, or in cell proliferation (Matsumoto & Wolffe, 1998); however, their entire function is still unclear. The import of transcription factors into the nucleus is not a constitutive process and appears to be modulated in response to external stimuli, cell cycle and development. Regulation of the subcellular localization of proteins involves the direct phosphorylation of the transported protein, masking of the nuclear localization signals, cytoplasmic retention by binding to an anchoring protein, and the interplay among these different mechanisms (Calkhoven & Ab, 1996; Garcia-Bustos et al., 1991). It has been proposed that the YB-1 protein migrates to the nucleus in response to certain stress signals (Koike et al., 1997). However, examination of the YB-1 sequence shows no obvious nuclear localisation sequence. The question arising from this issue is: How are Y-box proteins signaled to move into the nucleus? Is phosphorylation an effective factor for nuclear translocation? To address this issue, we considered possibilities to tag the protein to study compartmental changes and to investigate what region of the protein might be responsible for this translocation. A GFP fusion protein enabled us to observe the subcellular location and intracellular movements in vitro. Observations under the fluorescence microscope showed clearly a predominant expression of GFP-YB-1 and the truncated version YB-1(27 in the cytoplasm of Cef32 cells. Kokie and colleagues have reported that the C-terminal region of YB-1 might be important for cytoplasmic retention of YB-1. In their study, they demonstrated that expression of GFP-YB-1 with the C-terminus of YB-1 was located mainly in the cytosol, but GFP-YB-1deltaC with a larger deletion at the C-terminus of YB-1 (248-317) was located in the nucleus (Kokie et al., 1997), in contrast to our observation with a different deletion at the C terminus. The C-terminal domain of YB-1 does not have a nuclear export signal such as mitogen activated protein kinase kinase (MAPKK). Since this domain has been shown to be involved in the protein-protein interaction, it was hypothesized that YB-1 could be retained in the cytoplasm by binding to an anchoring protein (Ruzanov et al., 1999), as observed for the transcription factor NF- κ B interacting with I κ B in the cytoplasm (Verma et al., 1995).

The recruitment of YB-1 to the nucleus after exposure to UV irradiation or cytotoxic agents suggests that YB-1 itself protects the cells against the effects of genotoxic damage (Cohen et al., 2010). The association between nuclear YB-1 and resistance against cisplatin might be a useful predictive marker for cancer multidrug resistance (Yahata et al., 2002). Recently, an association of YB-1 intracellular localization with expression levels of P-gp (P-glycoprotein, which is able to dispose drugs from the cell via the cell membrane) has been proposed (Bargou et al., 1997). This P-glycoprotein is encoded by the MDR1 gene, which responsible for drug resistance of many tumors. Bargou have reported that nuclear localization of YB-1 is closely associated with MDR1 gene expression in a human breast cancer cell line and that P-gp levels were high in untreated primary breast cancers in which YB-1 was localized in the nucleus, but were low in the breast cancers where YB-1 was localized in the cytoplasm.

These results show that in untreated primary breast cancers, nuclear localization of YB-1 protein is associated with intrinsic multidrug resistance. It was shown that YB-1 has an important role in controlling MDR 1 gene transcription, and this finding provides a basis for the analysis of molecular mechanisms responsible for intrinsic multidrug resistance in human breast cancer. However, it remains unclear whether activated YB-1 directly affects MDR1 gene expression in response to genotoxic stress. In previous studies suggests that YB-1 may itself have a key role in self-defense signaling mechanisms, being initiated in response to cellular stress. The YB-1 proteins are also integral components of a eukaryotic redox signalling pathway (Duh et al., 1995), serving as a scaffold for the assembly of other factors. All this information indicates that these Y-box proteins may be a part of mammalian stress signal transduction mechanisms.

In our studies, we investigated the dependence of intercellular localization of human-YB-1 and chk-YB-1 in metastatic melanoma cells and chicken embryo fibroblasts, respectively, by stress factors such as UV irradiation and GaQ3 treatment. Furthermore, we demonstrated that the last 27 amino acids of the C-terminus are necessary for translocation of the protein into the nucleus, since this deletion (YB-1 Δ 27 (294-321)) correlated with an inhibition of nuclear translocation after UV irradiation. This sequence (27 amino acids) which is rich in SH groups is a suitable substrate for posttranslational modification, in particular phosphorylation by protein kinases at serine or threonine. The frequency of these amino acids increases the negative charge of the acidic sequences and probably improves the interaction of the C-terminal domain with other proteins (carrier proteins). Protein-protein interactions may be the cause of migration of chkYB-1 into the nucleus. Molecular intervention aiming at the inhibition of nuclear translocation of YB-1 may be a strategy to improve the efficacy of anticancer drugs.

In conclusion, nuclear localization of YB-1 is a cellular response to damaging agents such as UV irradiation or the investigational anticancer drug GaQ3. YB-1 itself may be worth investigating as a potential target in cancer therapy, in particular in combination with cytotoxic drugs in malignant melanoma.

5. Acknowledgment

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Melanoma is considered to be one of the most aggressive forms of skin neoplasms. Despite aggressive researches towards finding treatments, no effective therapy exists to inhibit the metastatic spread of malignant melanoma. The 5-year survival rate of metastatic melanoma is still significantly low, and there has been an earnest need to develop more effective therapies with greater anti-melanoma activity. Through the accomplishment of over 100 distinguished and respected researchers from 19 different countries, this book covers a wide range of aspects from various standpoints and issues related to melanoma. These include the biology of melanoma, pigmentations, pathways, receptors and diagnosis, and the latest treatments and therapies to make potential new therapies. Not only will this be beneficial for readers, but it will also contribute to scientists making further breakthroughs in melanoma research.

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