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Carprofen Induction of p75^{NTR} Dependent Apoptosis via the p38 MAPK Pathway in Prostate Cancer Cells

Fatima S. Khwaja[1], **Emily J. Quann**[2], **Nagarajan Pattabiraman**[3], **Shehla Wynne**[1], and **Daniel Djakiew**[1,3]

1*Department of Biochemistry and Molecular & Cellular Biology, Georgetown University Medical Center, Washington D.C.*

2*Immunology Program, Memorial Sloan Kettering Cancer Center, New York, NY*

3*Vincent T. Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington D.C.*

Abstract

The p75^{NTR} functions as a tumor suppressor in prostate epithelial cells, where its expression declines with progression to malignant cancer. Previously, we demonstrated that treatment with R-flurbiprofen or ibuprofen induced p75^{NTR} expression in several prostate cancer cell lines leading to p75^{NTR} mediated decreased survival. Utilizing the 2-phenyl propionic acid moiety of these profens as a pharmacophore, we screened an in silico data base of 30 million compounds and identified carprofen as having an order of magnitude greater activity for induction of p75^{NTR} levels and inhibition of cell survival. Prostate (PC-3, DU-145) and bladder (T24) cancer cells were more sensitive to carprofen induction of p75^{NTR} associated loss of survival than breast (MCF7) and fibroblast (3T3) cells. Transfection of prostate cell lines with a dominant negative form of p75^{NTR} prior to carprofen treatment partially rescued cell survival demonstrating a cause and effect relationship between carprofen induction of p75^{NTR} levels and inhibition of survival. Carprofen induced apoptotic nuclear fragmentation in prostate but not in MCF7 and 3T3 cells. Furthermore, siRNA knockdown of the p38 MAPK protein prevented induction of p75^{NTR} by carprofen in both prostate cell lines. Carprofen treatment induced phosphorylation of p38 MAPK as early as within 1 minute. Expression of a dominant negative form of MK2, the kinase downstream of p38 MAPK frequently associated with signaling cascades leading to apoptosis, prevented carprofen induction of the p75^{NTR} protein. Collectively, we identify carprofen as a highly potent profen capable of inducing p75^{NTR} dependent apoptosis via the p38 MAPK pathway in prostate cancer cells.

Keywords

Carprofen; p75^{NTR}; p38 MAPK; MK2; Apoptosis

Introduction

The p75 neurotrophin receptor (p75^{NTR}) is a 75 kD cell surface receptor glycoprotein that shares both structural and sequence homology with the tumor necrosis factor receptor super-family of proteins (1,2). Some of these proteins (e.g. p75^{NTR}, p55^{TNFR}, Fas, DRs3-6, EDAR) have similar sequence motifs of defined elongated structure (1) designated "death domains"

Address for Reprint Requests: Daniel Djakiew, Ph.D. Department of Biochemistry and Molecular & Cellular Biology Georgetown University Medical Center 3900 Reservoir Rd NW, Washington, D.C. 20057-1436 E-mail: djakiewd@georgetown.edu.

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based upon their apoptosis inducing function (2). In the human prostate the $p75^{NTR}$ protein is progressively lost in pathologic cancer tissues (3). The proportion of epithelial cells that have retained $p75^{NTR}$ expression in the organ confined pathological prostate is inversely associated with increasing Gleason's score and pre-operative serum PSA concentrations (4). In addition, immunoblot of human prostate epithelial cell lines derived from metastases exhibit a further reduction of $p75^{NTR}$ expression (5). Significantly, even though expression of the $p75^{NTR}$ protein is suppressed the gene encoding $p75^{NTR}$ appears intact in these prostate cancer cells (6). The loss of $p75^{NTR}$ expression is a result of a loss of mRNA stability (6). Following ectopic re-expression of the $p75^{NTR}$ in these cancer cells their rate of apoptosis increased (7). Additionally, the same ectopically expressing $p75^{NTR}$ cancer cells exhibited a retardation of cell cycle progression characterized by accumulation of cells in G1 phase with a corresponding reduction of cells in the S phase of the cell cycle (7). Consistent with these observations, the $p75^{NTR}$ has been characterized with both tumor suppressor and metastasis suppressor activity in prostate cancer cells (7,8).

Several studies have demonstrated that non-steroidal anti-inflammatory drugs (NSAIDs) are effective as anticancer agents for colorectal, breast, pancreatic cancer, squamous cell carcinoma of the head and neck, bladder, ovarian, lung and prostate cancer (9,10). With respect to prostate cancer, retrospective studies indicate that there is a significantly reduced risk of prostate cancer associated with regular use of NSAIDs (11-13). *In vivo* studies using rodents have indicated that NSAIDs can decrease the size of prostate tumors (14,15) and suppress the metastasis of prostatic cancer (14,16). There is no common mechanism of action underlying NSAIDs effectiveness against cancer cells. Some NSAIDs inhibit the cyclooxygenases (COX) that convert arachidonic acid to prostaglandins (17). Prostaglandins are thought to contribute to tumor growth by inhibiting apoptosis (18) and by inducing the formation of new blood vessels needed to sustain tumor growth (19). Hence, COX inhibition of prostaglandin synthesis could explain part of the anti-tumor activity of certain NSAIDs. However, NSAIDs can also inhibit tumor formation and growth of COX-null cell lines (20). In addition, NSAIDs that lack COX inhibitory activity can still have significant anticancer effects both *in vivo* (21) as well as *in vitro* (22). Similarly, growth of the DU-145 prostate cancer cell line that lacks expression of COX-1 and COX-2 is inhibited by NSAIDs (23). Interestingly, R-flurbiprofen and ibuprofen have been shown to induce $p75^{NTR}$ levels leading to apoptosis in prostate cancer cell lines (23). These profens activated the p38 MAPK pathway leading to stabilization of $p75^{NTR}$ mRNA and increased levels of $p75^{NTR}$ protein that subsequently induced apoptosis of the prostate cancer cells (24). In this report we utilized the 2-phenyl propionic acid moiety of the profens as a pharmacophore for an in silico search of related compounds and identified carprofen as having an order of magnitude greater activity for induction of $p75^{NTR}$ levels and inhibition of cell survival. Carprofen activity occurred through rapid phosphorylation of p38 MAPK which signaled through MK2 to increase levels of $p75^{NTR}$ protein and stimulate apoptosis in the prostate cancer cells.

Materials and Methods

Cell Lines and Culture Conditions

PC-3 and DU-145 prostate cell lines were obtained from the tissue culture core facility of the Georgetown University Lombardi Comprehensive Cancer Center. T24 bladder, MCF7 breast and 3T3 fibroblast cells were obtained from ATCC. All cell lines were maintained in Dulbecco's modified Eagle's medium (Mediatech Inc., Herndon, VA) containing 4.5g/L glucose and L-glutamine supplemented with antibiotic/antimycotic [100 units/mL penicillin G, 100 μg/mL streptomycin and 0.25 μg/mL amphotercin B (Mediatech Inc.)] and 10% fetal bovine serum (Sigma Chemical Co., St. Louis, MO). Cells were incubated in the presence of 5% CO_2 and air at 37°C.

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Drug Preparation, Treatment, Cell Lysis

Using 2-phenyl propionic acid as a pharmacophore we searched an *in silico* data base of approximately 30 million compounds from which nine aryl propionic acids were selected for further analysis. Stock solutions were prepared by dissolving each aryl propionic acid in dimethyl sulfoxide (Sigma Chemical Co. St. Louis, MO) at a concentration of 200 mM. Cells were seeded overnight at 70-80% confluency and were then treated for 48 hours at concentrations of 0, 20, 40, 60, 80 and 100 μM. Cell lysates of treated cells were prepared as previously described (7,8,23). The supernatant was retained and protein concentration was determined according to the manufacturer's protocol (BioRad Laboratories, Hercules, CA).

Immunoblot Analysis

Immunoblot analysis was performed as previously described (23). Membranes were incubated in the primary antibody: murine monoclonal anti-p75^{NTR} (1:2000, Upstate Cell Signaling Solutions, Lake Placid, NY), rabbit polyclonal phosphorylated p38 MAPK (1:1000), mouse monoclonal anti-p38α (1:1000 ; Cell Signaling Technology, Danvers, MA), or murine monoclonal anti-β-actin (1:5000, Sigma Chemical Co. St. Louis, MO). Membranes were subsequently incubated in goat-anti-mouse or goat-anti-rabbit horseradish peroxidase-conjugated secondary antibodies (BioRad Laboratories, Hercules, CA) at a dilution of 1:2000 and immunoreactivity visualized with a chemiluminescence detection reagent (Amersham Pharmacia Biotech, Piscataway, NJ). The positive control for p75^{NTR} expression was a whole cell lysate of A875 cells (Dr. Moses Chao, Cornell University, NY).

Cell Survival Assay with p75^{NTR} Dominant Negative Transfection and Hoechst Dye Nuclear Staining

An equal number of viable cells (2×10^3 cells/well) in 96 well culture plates (final volume of 100 μl culture medium per well) were incubated for 4 h at 37°C in a humidified atmosphere of 5% CO_2. Some cells were also transiently transfected with a p75^{NTR} dominant negative vector described previously (24,25,26). The ΔICD vector expresses a p75^{NTR} gene product with the intracellular domain (ICD) deleted. The ΔICD is an ecdysone-inducible p75^{NTR} vector, and therefore was co-transfected with the ecdysone receptor plasmid pVgRxR. The transfection was performed with LipofectAMINE reagent (Invitrogen Corporation, Carlsbad, CA) in serum-free medium for 6 hours, after which serum containing medium was added. After 18 subsequent hours, cells were incubated in 1 μM ponasterone A (Invitrogen Corporation, Carlsbad, CA) for 24 hours to drive expression of the dominant negative gene product. Following incubation with ponasterone A, cells were treated with carprofen (0 – 100 μM) for 48 hours and relative cell survival was determined with MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) labeling reagent (final concentration 0.5 mg/ml, Roche Diagnostics Corporation, Indianapolis, IN). Subsequently, cells were incubated overnight with 100 μl of solubilization solution per well and the samples quantified at 570 nm using a micro titer plate reader (BioRad Laboratories, Richmond, CA). Hoechst dye nuclear (DNA) staining to identify apoptotic nuclei was conducted as described previously (25). PC-3, DU-145, MCF-7 and 3T3 cells were treated for 48 hours with carprofen and then fixed in 10% formalin (Electron Microscopy Sciences, Hatfield, PA). Some cells were transfected with the ΔICDp75^{NTR} plus ponasterone A prior to carprofen treatment.

siRNA Transfection

Cells were transfected for 72 hours with non-targeting siRNA or siRNA specific for p38α (J-003512-20), (Dharmacon RNA Technologies, Lafayette, CO) at final concentrations of 100 nM according to the manufacturer's protocol. Transfection reagent DharmaFECT 1 was used for DU-145 cells, and DharmaFECT 2 was used for PC-3 cells (Dharmacon RNA

Technologies, Lafayette, CO). After transfection, the cells were treated with carprofen for 48 hours, followed by determination of p75^{NTR} protein expression.

MK2 Dominant Negative Transfection

PC-3 and DU-145 cells were transiently transfected with a MK2 dominant negative vector (MK2-K76R) described previously (27). The transfection was performed with LipofectAMINE reagent (Invitrogen Corporation, Carlsbad, CA) in serum-free medium for 6 hours, after which serum containing medium was added for 24 hours to allow expression of the dominant negative gene product. Cells were treated with carprofen (100 μM) for 48 hours and expression of p75^{NTR} protein determined by immunoblot with mouse monoclonal anti-p75^{NTR} (1:2000; Millipore, Billerica, MA) (1:2000).

Statistical Analysis

The statistical differences between data sets and/or means were analyzed by ANOVA or the Mann-Whitney test using the Prizm program (Graph Pad Software) and the data expressed as the mean ± SEM. Data was considered statistically significant when $p \leq 0.05$.

Results

Carprofen Exhibits Superior Efficacy of the Aryl Propionic Acids to Induce p75^{NTR} Levels Associated with Cell Specific Decreased Survival

Analysis of the 2-phenyl propionic acid pharmacophore homology search identified nine aryl propionic acids that were screened for activity to induce expression of p75^{NTR} protein in PC-3 and DU-145 human prostate cancer cells. Initially, the PC-3 and DU-145 cell lines were selected since they are the only two prostate tumor cell lines included in the NIH DTP anticancer drug discovery program. The immunoblots demonstrating activity of each compound to induce p75^{NTR} were placed in rank-order (Figure 1). In both cell lines, carprofen exhibited superior efficacy for induction of p75^{NTR} expression at a concentration of 100 μM or less compared with all other aryl propionic acids examined (Figure 1). At lower concentrations, carprofen selectively induced expression of p75^{NTR} protein at approximately 40 μM and above in PC-3 and DU-145 prostate cancer cells, as well as in the T24 bladder cancer cell line, but not in the MCF-7 breast cancer cell line or the 3T3 fibroblast cell line (Figure 2A). The T24 bladder cancer cell line was included as a positive control since they were previously shown to be sensitive to profen (ibuprofen, R-flurbiprofen) induced p75^{NTR}-dependent decreased survival, whereas MCF-7 and 3T3 cells were included as negative controls since they were previously shown not to be sensitive to profen (ibuprofen, R-flurbiprofen) induced decreased survival (25).

Carprofen treatment selectively decreased the survival of cells in rank-order with PC-3 and DU-145 prostate cancer cells exhibiting greatest sensitivity to dose-dependent decreased survival followed by the T24 bladder cancer cells, and with MCF-7 and 3T3 fibroblasts the least sensitive to carprofen induced decreased survival (Figure 2B). Significantly, there was a strong association between the dose-dependent induction of p75^{NTR} levels (Figure 2A) and decreased survival of specific cell types following carprofen treatment (Figure 2B).

Carprofen Induced Decreased Prostate Cancer Cell Survival is Dependent on p75^{NTR}

In order to establish a causal relationship between carprofen induction of p75^{NTR} protein expression and inhibition of cell survival we utilized a ponasterone A inducible expression vector for p75^{NTR} that exhibits a deletion of the intracellular death domain (ΔICDp75^{NTR}) shown to function as a dominant negative antagonist of the intact p75^{NTR} gene product (23-26). The treatment of both PC-3 and DU-145 cells with carprofen or carprofen plus

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ponasterone A inhibited cell survival in a dose-dependent manner (Figure 3). However, both PC-3 and DU-145 cell lines induced with ponasterone A to express ΔICDp75^{NTR} exhibited a significant ($p < 0.001$) partial rescue from carprofen-mediated inhibition of cell survival relative to carprofen treated ΔICDp75^{NTR} cells in the absence of ponasterone A (Figure 3). Subsequently, we examined Hoechst stained nuclear morphology to identify fragmented nuclei typical of apoptotic cells with the exception of T24 bladder cells for which we have previously demonstrated profen induced apoptotic nuclear fragmentation (25). Treatment of the two prostate cancer cell lines (DU-145, PC-3) with carprofen induced a dose-dependent (0 – 100 μM) fragmentation of nuclei (Figure 4). As negative controls, the MCF7 and 3T3 cells that were not induced by carprofen to express p75^{NTR} (Figure 2A) did not undergo carprofen dependent apoptotic nuclear fragmentation (Figure 4). Expression of the ΔICDp75^{NTR} dominant negative vector prior to carprofen treatment partially rescued nuclear fragmentation in the PC-3 and DU-145 prostate cells, whereas the MCF-7 and 3T3 negative control cells did not exhibit fragmented nuclei (Figure 4).

Carprofen Induction of p75^{NTR} occurs via the p38 MAPK Pathway

An earlier study from our laboratory (24) implicated the aryl propionic acids, R-flurbiprofen and ibuprofen, in the induction of p75^{NTR} via the p38 MAPK pathway. Since carprofen, an aryl propionic acid, exhibits an order of magnitude greater potency (Figure 2A) than R-flurbiprofen and ibuprofen for the induction of p75^{NTR} expression levels (23,24) we examined the effect of siRNA knockdown of the p38α MAPK isoform on p75^{NTR} levels following treatment with carprofen. We previously demonstrated that p38α MAPK is the predominant isoform expressed in PC-3 and DU-145 cells (24). Whereas treatment with carprofen induced p75^{NTR} expression levels, transfection of prostate cancer cells with p38α siRNA prior to carprofen treatment prevented induction of p75^{NTR} relative to untransfected cells or cells transfected with non-targeting siRNA (Figure 5A).

Since the p38 MAPK is activated by phosphorylation we determined the phosphorylation status of p38 MAPK at several time points in PC-3 and DU-145 cells following treatment with carprofen. In both cell lines, carprofen treatment stimulated rapid phosphorylation of p38 MAPK as early as within one minute of treatment, and subsequently led to the sustained activation of the p38 MAPK pathway that could be observed even 8 hours following treatment of each cell line (Figure 5B).

Since the MK2 kinase is downstream of p38 MAPK and was previously shown to be involved in profen induction of p75^{NTR} (24) we used a dominant negative expression vector for MK2 to determine involvement in carprofen induction of p75^{NTR}. Treatment with carprofen alone induced expression of p75^{NTR} in both PC-3 and DU-145 cells, whereas transfection of dnMK2 prior to carprofen treatment decreased the induction of p75^{NTR} (Figure 6).

Discussion

Carprofen is a propionic acid NSAID that induced p75^{NTR} levels in prostate cancer cell lines with an order of magnitude greater efficacy than the related propionic acid NSAIDs, R-flurbiprofen and ibuprofen (23). Concomitant with carprofen's superior efficacy to induce levels of p75^{NTR} was its activity to inhibit cell survival via apoptosis. Our previous studies have demonstrated a strong cause and effect relationship between induced levels of p75^{NTR} and induction of apoptosis in cancer cell lines (23,25). When expression levels are induced, p75^{NTR} appears to be a robust marker of drug induced apoptosis (23,25). Since carprofen exhibited some degree of cell specific induction of p75^{NTR} associated apoptosis, we focused on the PC-3 and DU-145 prostate cancer cell lines which were most responsive to carprofen treatment, and coincidently are the only two prostate cancer cell lines included in the NIH DTP anticancer drug discovery program, due to their well characterized aggressive phenotype.

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Hormone responsive prostate cells were intentionally not included in these studies in order to maintain a focus on potential therapeutics of prostate tumor cells with phenotypes refractory to hormone ablation treatment consistent with poor prognosis. Using the prostate cancer cell lines, PC-3 and DU-145, most responsive to carprofen, we showed that a dominant negative antagonist of $p75^{NTR}$ (ΔICD$p75^{NTR}$) partially rescued carprofen induced inhibition of cell survival, thereby confirming a cause and effect relationship between carprofen induction of $p75^{NTR}$ levels and $p75^{NTR}$ induction of apoptosis. Partial rather than complete rescue may be attributed to assay conditions or additional effects of carprofen independent of $p75^{NTR}$.

In prostate cancer cell lines re-expression of $p75^{NTR}$ induces modifications to several down stream signal transduction cascades leading to apoptosis. Initially, $p75^{NTR}$ expression down regulates components of the NFκB and JNK pathways preventing nuclear translocation of both these pro-survival transcriptional effectors (28). Expression of $p75^{NTR}$ also retards cell cycle progression through accumulation of cells in G1 at the expense of S phase cells (7,26). Down regulation of cyclin/cdk holoenzyme components cyclin E, cyclin A, cdk2 and cdk6 contribute to hypo-phosphorylation of retinoblastoma (Rb), along with elevated levels of $p16^{INK4a}$ in the $p75^{NTR}$ induced cytostatic cells (26). Re-expression of $p75^{NTR}$ also induces elevated expression of the retinoic acid receptor, RAR-β, and retinoid X receptors (RXRα, RXR-β) during partial re-differentiation of PC-3 cells that may also contribute to cytostasis (29). Evidence for $p75^{NTR}$ dependent activation of extrinsic apoptosis in prostate cells has been limited to caspase-8 reductions in RIP, an adaptor protein that interacts with the intracellular domain of $p75^{NTR}$ (28). Evidence for $p75^{NTR}$ dependent activation of the intrinsic mitochondrial pathway includes an increase in pro-apoptotic effectors, Smac, Bax, Bak and Bad and conversely a decrease in the pro-survival effector, Bcl-xl (26) leading to a reduction in XIAP and cleavage of caspase-9 and caspase-7 followed by PARP cleavage and nuclear fragmentation in PC-3 cells (26). Hence, re-expression of $p75^{NTR}$ appears to promote partial re-differentiation, cell cycle arrest and apoptosis in prostate cancer cells thereby providing a rationale for investigation of compounds that may be used for $p75^{NTR}$ dependent therapeutics.

Prostate cancer cells evade the apoptotic effects of $p75^{NTR}$ expression by loss of $p75^{NTR}$ mRNA stability with concomitant suppression of $p75^{NTR}$ protein levels (6). Conversely, R-flurbiprofen and ibuprofen stabilize $p75^{NTR}$ mRNA with concomitant expression of $p75^{NTR}$ protein (24) and induction of apoptosis (23) through the p38 MAPK pathway (24). Indeed, abundant evidence has been reported for the involvement of p38 MAPK in apoptosis induced by a variety of agents such as the profen NSAIDs (23,24), Fas ligation (30) and NGF withdrawal (31). The later is significant since NGF ligation to the $p75^{NTR}$ acts as a survival signal in prostate cancer cells (26). Conversely, a relative absence of NGF, either by ligand withdrawal, or by up-regulation of $p75^{NTR}$ protein to levels that initially bind residual ligand and then to higher levels that result in unbound (no ligand) $p75^{NTR}$ acts as a stimulus of apoptosis in prostate cancer cells (23,26,28). From a potential therapeutic perspective this mechanism of $p75^{NTR}$ dependent apoptosis has the appeal that agents, such as the profens, that elevate $p75^{NTR}$ levels have the same effect as ligand withdrawal leading to apoptosis of cancer cells. Activation of the p38 MAPK signal transduction pathway by carprofen was rapid, within 1 minute, suggesting that carprofen is interacting with a molecule highly proximal to p38 MAPK. The observation that p38 MAPK knockdown prevented carprofen induction of $p75^{NTR}$ levels confirms this pathway as a mechanism responsible for $p75^{NTR}$ regulation. We recently reported similar observations for R-flurbiprofen and ibuprofen activation of p38 MAPK up-regulation of $p75^{NTR}$ dependent apoptosis in prostate cancer cells (24). In this pathway MK2 directly binds to the p38α isoform of MAPK during activation (32). Expression levels of MK2 are relatively robust in both PC-3 and DU-145 prostate cancer cells (24). Indeed, dominant negative antagonism of MK2 prevented carprofen induction of $p75^{NTR}$ levels in prostate cancer cells. These observations suggest that carprofen initiates $p75^{NTR}$ dependent apoptosis through a similar p38 MAPK signal transduction pathway to that of R-flurbiprofen and ibuprofen, albeit

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at an order of magnitude lower concentration of drug. Additional studies of this mechanism may lead to more potent compounds that induce p75^{NTR}-dependent apoptosis of prostate cancer cells.

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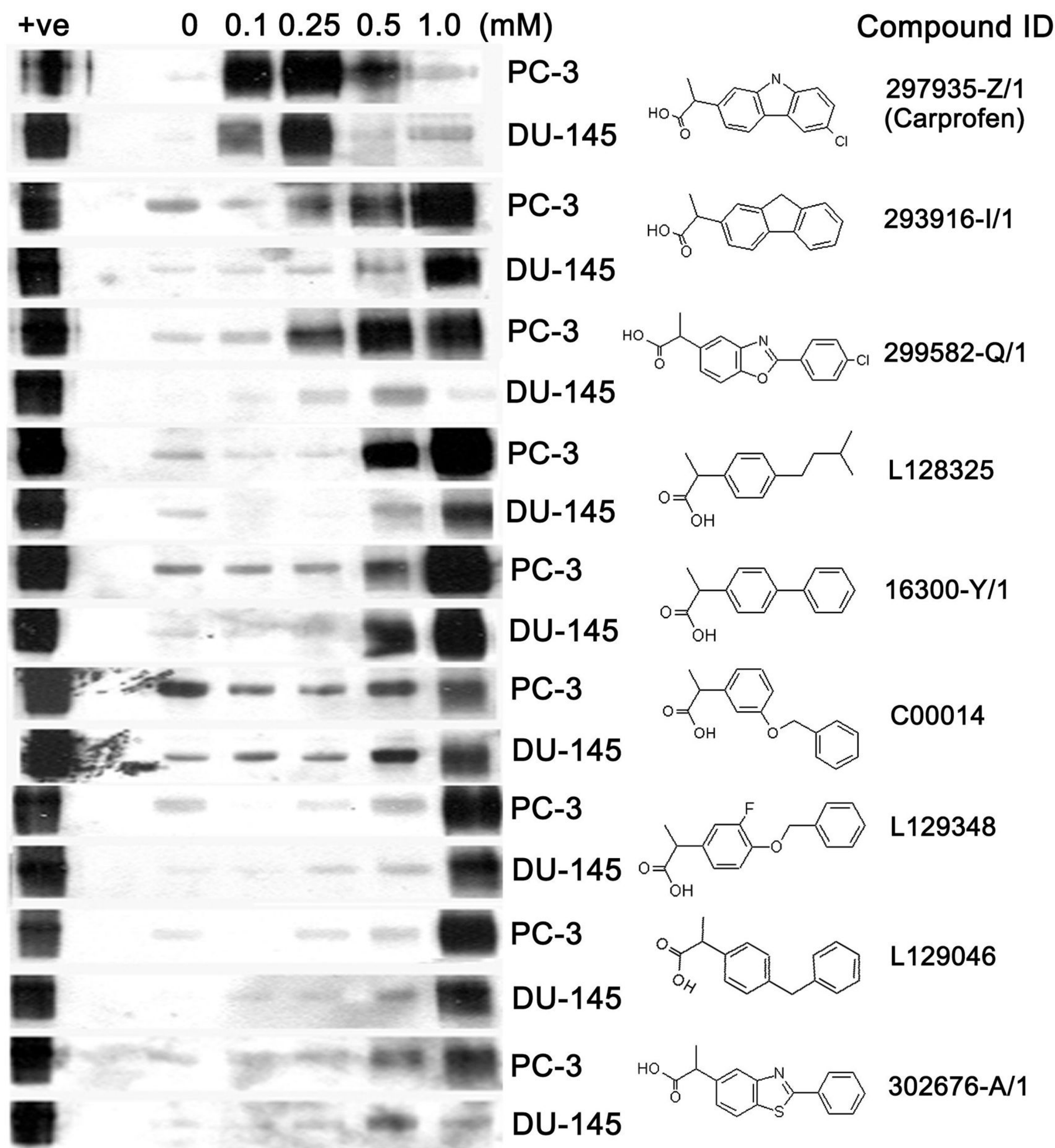

Figure 1.
Immunoblots of $p75^{NTR}$ levels in PC-3 and DU-145 cells following 48 hour treatment with 0, 0.1, 0.25, 0.5, and 1.0 mM of drug. The compound identification (ID) of each drug is given adjacent to its chemical structure. The A875 melanoma cell line was used as a positive control (+ve) for $p75^{NTR}$ expression.

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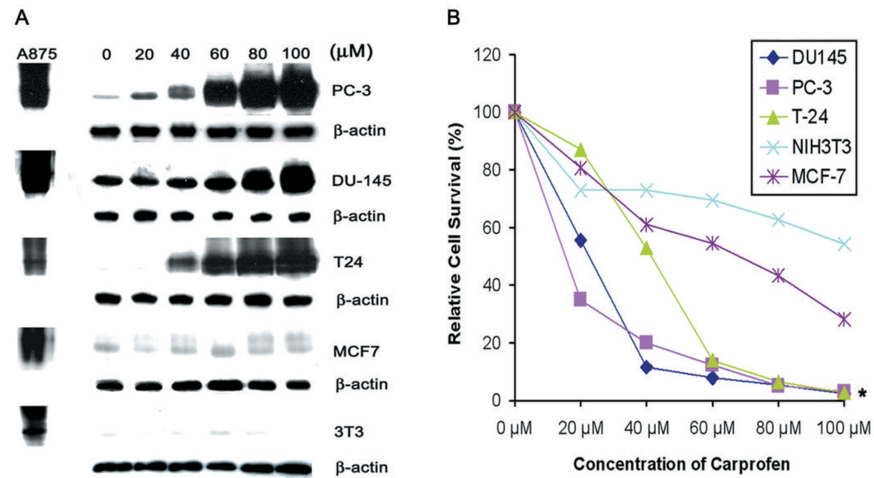

Figure 2.

A. Immunoblots of $p75^{NTR}$ levels with corresponding α-actin loading controls in PC-3 and DU-145 prostate cancer cells, T24 bladder cancer cells, MCF-7 breast cancer cells and 3T3 fibroblasts after 48 hour treatment with 0, 20, 40, 60, 80 and 100 M carprofen. A875 cell lysates were used as positive controls (+ve) for $p75^{NTR}$ expression. **B.** A MTT cell survival assay of PC-3 and DU-145 prostate cancer cells, T24 bladder cancer cells, MCF-7 breast cancer cells and 3T3 fibroblasts following 48 hour treatment with 0, 20, 40, 60, 80 and 100 μM carprofen. *$p<0.01$

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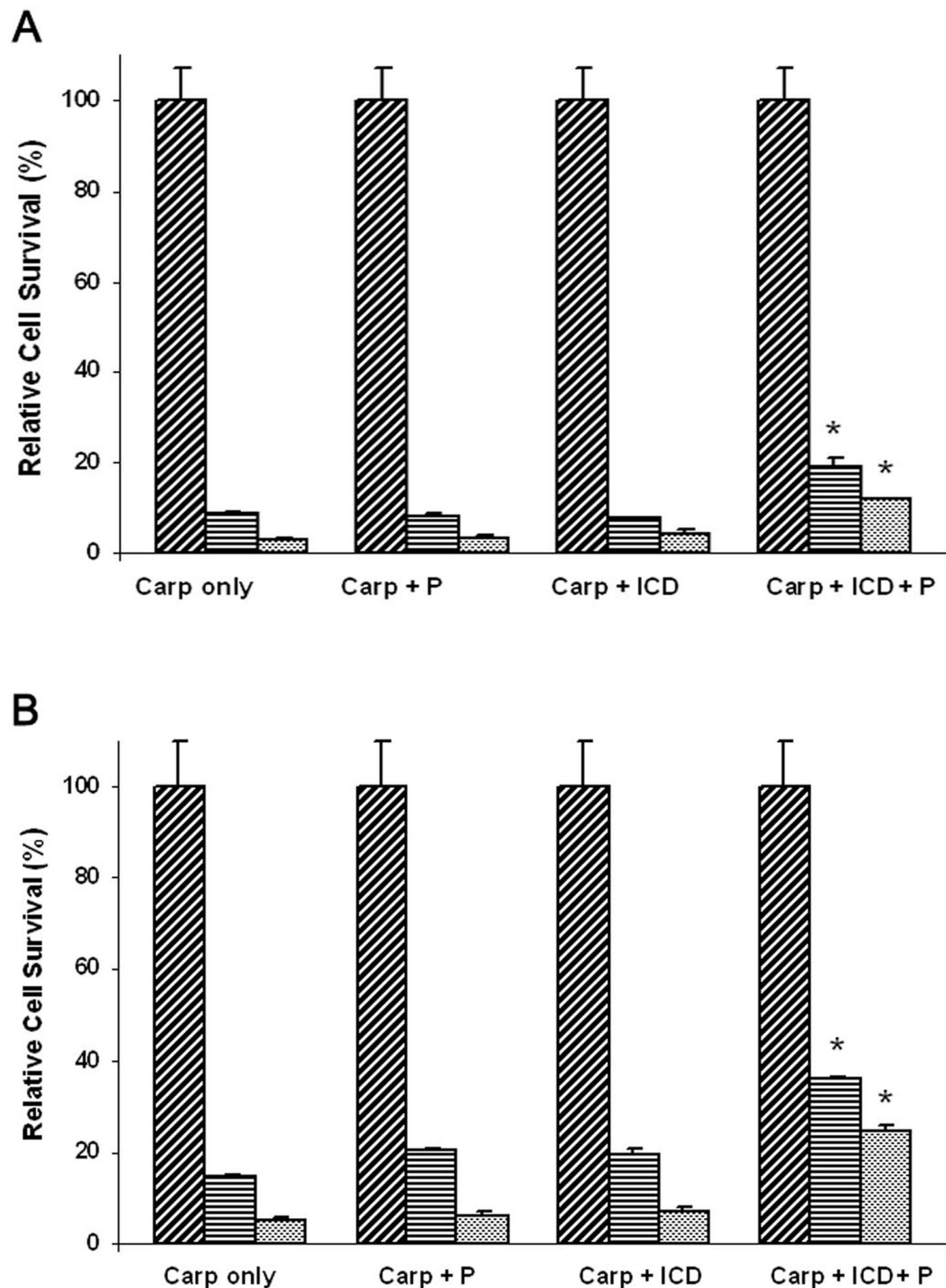

Figure 3.

PC-3 (**A**) and DU-145 (**B**) cell survival analysis by MTT assay following 48 hour treatment with 0 μM (cross-hatched), 50 μM (diagonal), or 100 μM (stippled) carprofen (Carp). Prior to treatment, cells were co-transfected with a ponasterone A-inducible ecdysone receptor plasmid pVgRxR and ΔICDp75^{NTR} (ICD). Following transfection, cells were incubated in serum containing medium for 18 hours, and then incubated in 1 μM ponasterone A (P) for 24 hours to drive expression of the dominant negative gene products (ICD). Results are expressed relative to the control (0 μM). *, $P < 0.001$

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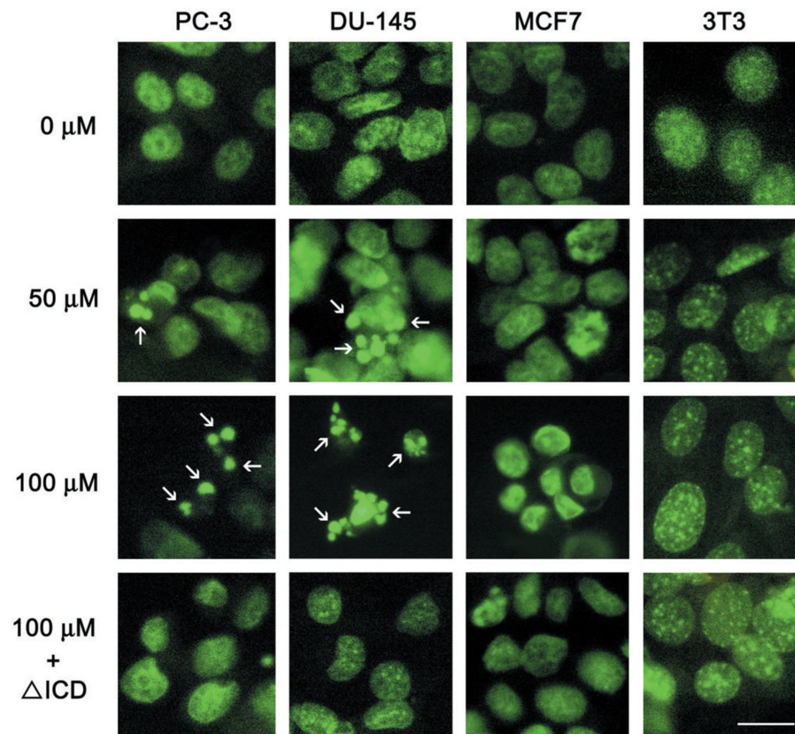

Figure 4.

Detection of apoptotic nuclei (arrows) by Hoechst staining of PC-3 and DU-145 prostate cancer cells, MCF-7 breast cancer cells and 3T3 fibroblasts following treatment with 0, 50 or 100 μM carprofen for 48 hr. Prior to treatment, some cells were co-transfected with a ponasterone A-inducible ecdysone receptor plasmid pVgRxR and ΔICDp75^{NTR} (ICD). Following transfection, cells were incubated in serum containing medium for 18 hours, and then incubated in 1 μM ponasterone A (P) for 24 hours to drive expression of the dominant negative gene products and then treated with 100 μM carprofen for 48 hr. Scale bar = 5 μM

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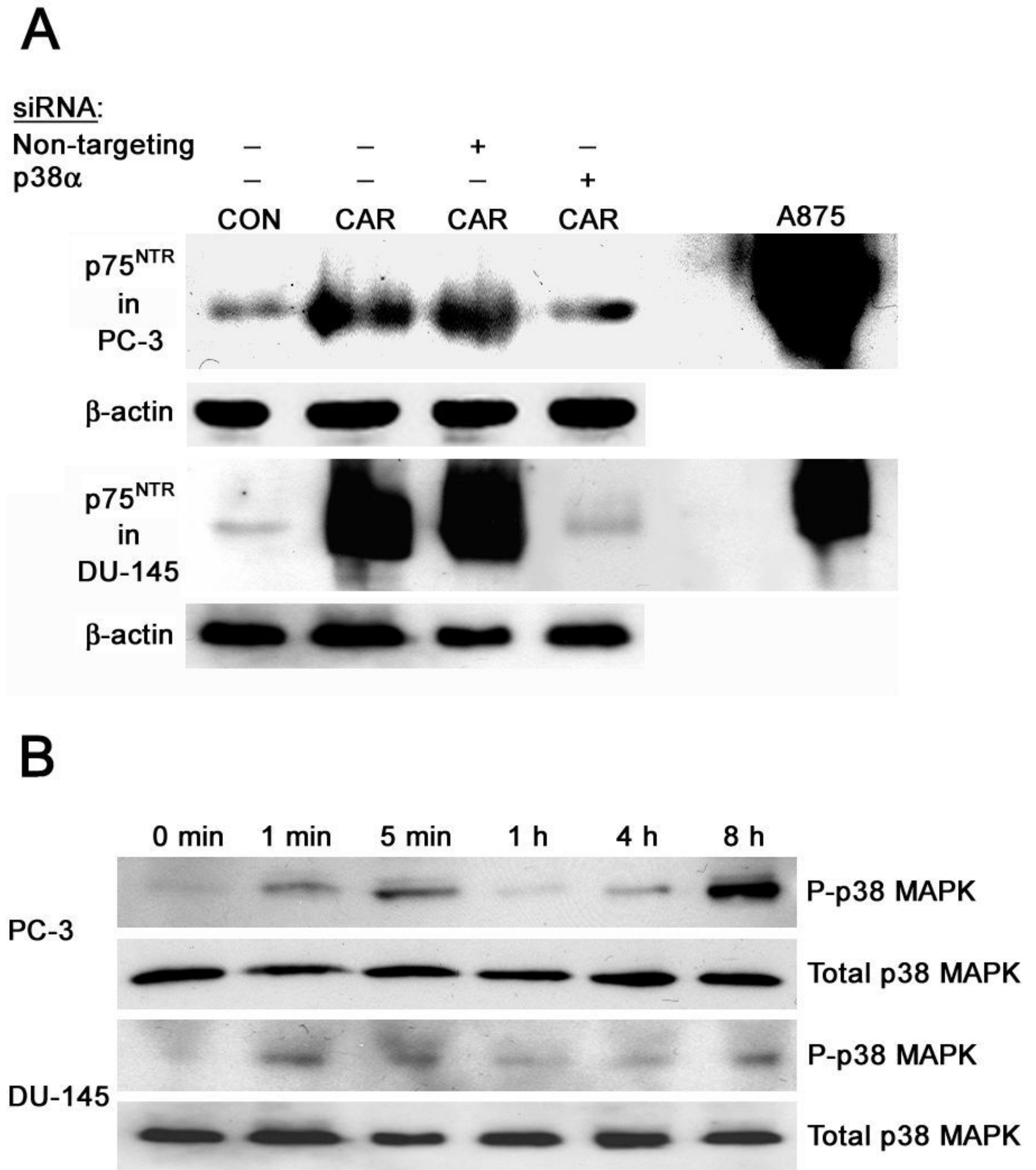

Figure 5.

A. Knockdown of p38 MAPK prevents induction of p75^{NTR} by carprofen. PC-3 and DU-145 cells were transfected with non-targeting siRNA or siRNA for p38α for 72 hours. Following transfection, cells were treated with 100 μM carprofen (CAR), or DMSO vehicle control (CON) and the cell lysates used for immunoblot analysis. A875 cell lysates were used as a positive control for p75^{NTR} expression. β-actin (β-act) was used as the loading control. **B.** Activation of the p38 MAPK pathway by carprofen. PC-3 and DU-145 cells were treated with 100 μM carprofen for 0 minutes, 1 minute, 5 minutes, 1 hour, 4 hours, or 8 hours. Cell lysates were prepared for immunoblot analysis using antibodies to phosphorylated p38 MAPK (P-p38). Blots for P-p38 MAPK were stripped and reprobed for total p38 MAPK.

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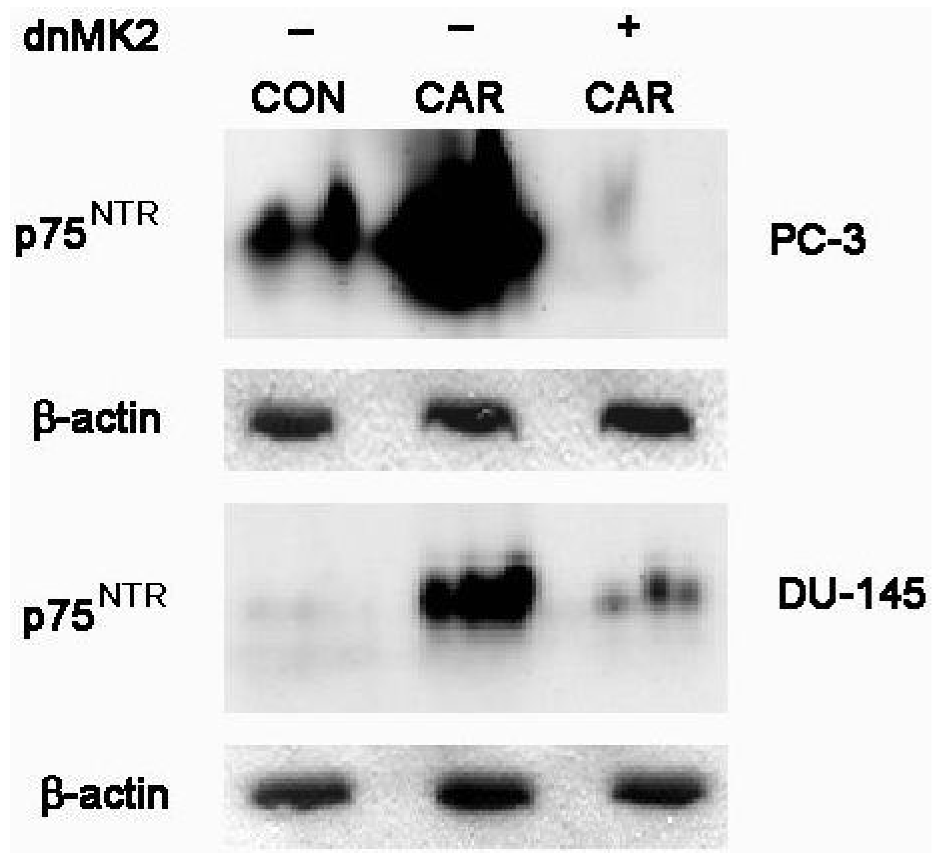

Figure 6.
PC-3 and DU-145 cells were transfected with dominant negative MK2 (dnMK2), after which serum containing medium was added for 24 hours to allow expression of the dominant negative gene product. Cells were treated with 100 μM carprofen (CAR) for 48 hours. Cell lysates were collected for immunoblot analysis of p75^{NTR} levels. β-actin was used as the loading control.