Apoptotic Effects of Novel Dithiocarbamate Analogs of Emetine in Prostate Cancer Cell Lines

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Abstract. *Background/Aim: Prostate cancer is one of the leading causes of death in American males. Emetine, a naturally-derived alkaloid from the Ipecacuanha plant, has been shown to have potential for anti-tumorigenic effects for cancer treatments. The objective of this study was to characterize novel emetine dithiocarbamate (EMTDTC) analogs for potent anti-tumorigenic activity with minimal toxicity to normal prostate cells and identify targeted apoptotic regulatory genes. The leading key compounds, EMTDTC-55 and EMTDTC-56 were studied. Materials and Methods: Established methods of cell flow cytometry were used to analyze apoptotic potential in prostate cancer cell lines (DU145, PC3 and LNCaP) and real time-polymerase chain reaction (PCR) for identifying key genes mediating apoptosis. Results: The effect of EMTDTC-55 on DU145, LNCaP and PC3 revealed significant anti-tumorigenic activities. Both compounds showed highly significant apoptotic potential on days 3 and 5 in the prostate cancer cells. Key apoptotic genes were differentially regulated suggestive of cell-cycle arrest and apoptotic induction in androgen-independent cell lines, DU145 and PC3, by both compounds. However, in the androgen-dependent cell line LNCaP, cells were marginally affected by EMTDTC-55, but significant apoptosis was observed by EMTDTC-56 leading to cell-cycle arrest. Conclusion: Both dithiocarbamate compounds EMTDTC-55 and EMTDTC-56 have significant chemotherapeutic potential in moderately metastatic DU145 and highly metastatic PC3 cells.*

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The history of emetine dates back centuries ago, traditionally used in herbal medicine and later introduced to Western medicine almost 400 years ago. It has stood the test of time for its promising therapeutic properties as an ipecac alkaloid in the treatment of certain dysenteries; its effectiveness as emetic and expectorant has been well-recognized as early as the 17th century (1).

Evidence of emetine's anti-tumorigenic activity was first identified in 1918 (2) and was investigated in Phase I and II clinical cancer trials in some solid tumors 40 years ago (3-8); however, emetine disappeared from cancer therapy development by 1975 (3). The high cytotoxicity of emetine is due to the inhibition of protein biosynthesis in eukaryotic ribosomes (1, 9) and its interaction with DNA (3). There are several publications suggesting the induction of apoptosis by emetine in U937 cells (10, 11), A549-S cells (12) and rat hepatocytes (3, 13). Thus, emetine could be a suitable cytotoxic agent in cancer therapy, *e.g.* to overcome multi-drug resistance or to take advantage of synergistic effects in order to minimize side-effects due to the high dosage of other cytotoxic agents (3).

Research has shown that the main role of emetine is to inhibit protein synthesis by binding to the 40S ribosomal subunit and inhibiting translocation in eukaryotic cells (14). The effects of emetine on protein synthesis have been extensively reported in various types of tissues like cardiac, muscle and brain tissues (15-18). Efforts in drug development of emetine have been limited because it is a protoplasmic poison that impacts tissues of the muscle, nervous system, heart, vascular, gastro-intestinal and skeletal systems (19). There have been recent efforts directed at developing emetine analogs with better therapeutic indices (20).

The main objective of the present study was to investigate whether novel dithiocarbamate analogs of emetine with an altered functional moiety have potent anti-tumorigenic activity while offering reduced toxicity to normal prostatic cells. In addition, efforts have been made to elucidate the

gene regulatory pathways targeted by the compound, which mediate cell survival, proliferation and apoptosis. Therefore, this study offers insight that may improve current regimens for efficacious treatment of prostate-derived metastasized tumors, as well as reducing chemotherapeutic side-effects.

Materials and Methods

Compounds and reagents. The emetine dithiocarbamate analogs (EMTDTC), compounds EMTDTC-55 and EMTDTC-56, were designed and synthesized as previously reported (20, 21). 3-[4,5-dimethylthiozal-2-yl]-2,5,-diphenyltetrazolium bromide (MTT), trypan blue dye and all other reagents were purchased from Sigma Chemical Company (St. Louis, MO, USA), Annexin V-APC dye and buffer were obtained from BD Pharmagen (San Diego, CA, USA) and Calcein-AM from Invitrogen (Grand Island, NY, USA).

Cell culture. Prostate cancer cell lines were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA): stock cultures of human androgen-independent prostate cancer cell lines DU145 (derived from brain metastasis), PC3 (derived from metastasis to bone), androgen-dependent LNCaP (metastasis to supraclavicular lymph node) and the normal prostate derived cell line PNT1A, were maintained as follows. Cells were incubated in 75 cm^2 flasks in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Invitrogen) and 1% penicillin- streptomycin (Invitrogen) at 5% CO_2/95% air at 37˚C. Upon reaching 80% confluence, the cells were trypsinzed, stained with 0.2% trypan blue dye and counted using a hemocytometer.

Determination of apoptotic cells using flow cytometry. To quantify the compound inducing apoptosis (EMTDTC-55 *vs*. EMTDTC-56), cells were double stained by Calcein-AM and Annexin V-APC and positivity was assessed by cell flow cytometry. The four prostate cell lines were seeded at a density of 1×10^5 cells per well in 12-well plates and incubated at 5% CO_2/95% air at 37˚C. Untreated cells were used as controls, while experimental wells were treated with EMTDTC-55 and EMTDTC-56 at concentrations corresponding to the half maximal inhibitory concentration IC_{50} as obtained from a MTT assay (data not shown). Cells were harvested on day 1, 3 and 5 and readings were performed in triplicate. Plates were centrifuged at 1,100 × *g* for 5 min. The floating cells in media was transferred to 1.5 ml polymerase chain reaction (PCR) tube. The wells were washed with 1X PBS, centrifuged again and trypsinzed with 200 μl Trypsin/0.05%EDTA for 2 min and transferred to the aliquot tube. The tubes were centrifuged at 1,100 × *g* for 5 min and washed with cold 1X PBS. Care was taken to assure that both floating and attached cells were obtained for apoptosis analysis. Cells were re-suspended in 500 μl 1X PBS of 1 μM Calcein-AM (Invitrogen) and stained for 30 min for detection of green fluorescence at 585 nm. After centrifugation the supernatant was decanted and cells were re-suspended in 100 μl of 1X Annexin V Binding buffer (BD Pharmagen) (0.1M HEPES buffer (pH7.4), 1.4M NaCl and 25 mM $CaCl_2$ solution). Five μl of Annexin V-APC solution (BD Pharmagen) was added, cells were gently vortexed and incubated at room temperature (25˚C) for 15 min in the dark. Ten μl of 0.05% EDTA was added to each tube to prevent cells from clumping during analysis. Total sample volume was brought up to 500 μl with 1X PBS (~400 μl). FACS analysis was performed within 1 hour using BD FACS Calibur™ (BD Biosciences, San Jose, CA, USA). The following controls were also set up for each of the four cell lines for comparison and defining quadrants for the analyser: (i) unstained cells, (ii) cells stained with Calcein only and (iii) cells stained with Annexin V- APC only. Double staining was performed to help distinguish between viable, early apoptotic, late apoptotic and necrotic cells. The cell flow cytometry data were expressed in terms of percent live cells on days 1, 3 and 5 with respect to the control using Graphpad InStat, version 6.0 (Graphpad Software Inc, San Diego, CA, USA).

Table I. *Fold regulation changes in key apoptotic genes. Real-time PCR analysis of PNT1A, DU145, PC3 and LNCaP treated with EMTDTCs. Gene expression profiles of DU145, PC3 and LNCaP were normalized to PNT1A.*

Fold regulation changes in key apoptotic genes by EMTDTC-55

Genes	PNT1A	Normalized DU145	Normalized PC3	Normalized LNCaP
BAX	–1.347	–0.406	0.344	0.950
BCL2	–1.320	3.320	3.384	–1.314
CDKN1A	–1.021	5.021	9.054	–1.168
CDKN2A	1.301	1.815	–2.453	1.314
FASLG	3.387	2.349	–4.757	–0.350
NFKB1	–1.007	4.418	6.767	–1.120
PMEPA1	2.621	4.490	20.101	0.253
TNF	–4.287	–10.033	3.212	0.561
TP53	–2.497	3.911	4.186	0.820
VEGFA	1.000	39.224	2.473	2.180

Fold regulation changes in key apoptotic genes by EMTDTC-56

Genes	PNT1A	Normalized DU145	Normalized PC3	Normalized LNCaP
BAX	–1.370	0.316	4.136	0.147
BCL2	1.271	2.910	11.617	0.757
CDKN1A	1.460	1.150	7.102	–6.558
CDKN2A	1.576	0.619	–2.627	–0.310
FASLG	6.046	–3.998	–4.719	–3.584
NFKB1	1.609	1.001	5.203	–0.308
PMEPA1	2.596	14.601	50.776	–4.925
TNF	–4.039	–30.401	14.012	–20.551
TP53	–2.076	3.586	330.178	–48.486
VEGFA	–1.269	47.603	5.732	4.177

Identifying apoptotic genes regulated by EMTDTCs using real-time PCR. Total RNA extraction and isolation were performed as per the manufacturer's protocol using the RNeasy Mini Kit (Qiagen, address). The four cell lines were grown in individual 25 cm^2 flasks, as previously stated, and treated with EMTDTC-55 and EMTDTC-56 at the corresponding IC_{50} dose. After 24 hours following treatment, the cells were harvested by disaggregation in 2 ml Trypsin/0.05% EDTA, transferred to 15-ml tubes and pelleted at 1, 100 × *g* for 5 min at 4˚C. cDNA was prepared using the RT2 first strand synthesis kit (SA Biosciences, Valencia, CA, USA). Quantitative real-time PCR was performed using primers specific to 10 genes regulating apoptosis (*BAX, BCL2, CDKN1A, CDKN2A, FASLG, NFKB1, PMEPA1, TNF,*

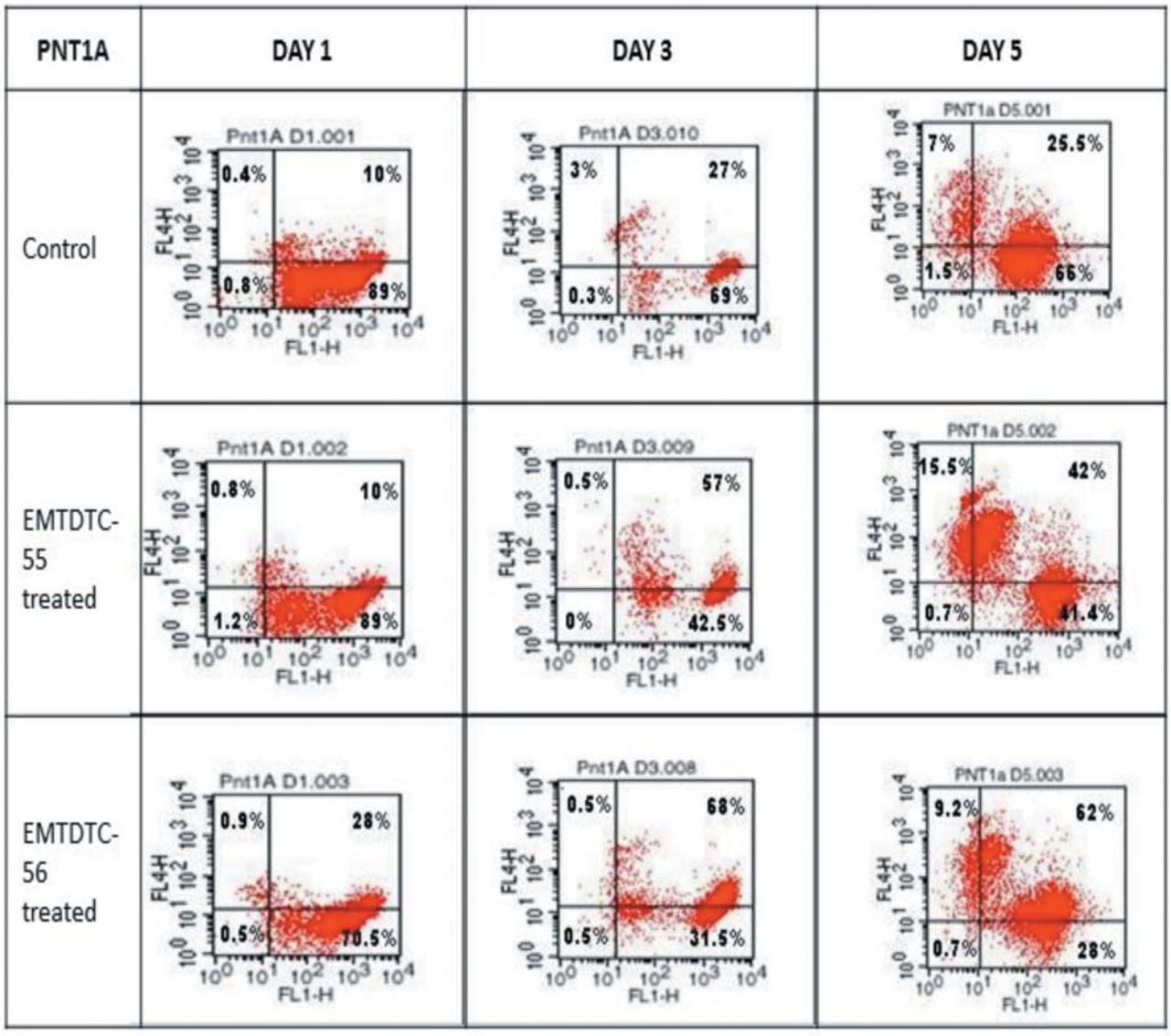

Figure 1. *Apoptosis in PNT1A cells after treatment with EMTDTCs. Flow cytometry performed on PNT1A cells treated with EMTDTCs on Day 1, 3 and 5. The FL1-H (X-axis) is the green channel and the FL4-H (Y-axis) is the red channel by FACS analysis. Quadrants denote live (lower right), early apoptotic (upper right), late apoptotic (upper left) and necrotic (lower left) cells.*

TP53 and *VEGFA*) using the RT^2 SYBR Green master mix (SA Biosciences, Valencia, CA, USA) on a Stratagene Mx3500p (Agilent Technologies, Santa Clara, CA, USA). Samples were normalized using *GAPDH* and β-actin (*ACTB*).

Results

Emetine dithiocarbamate analogs induce apoptosis. Apoptosis was analyzed in prostate cancer cell lines DU145, PC3 and LNCaP, as well as in normal prostate-derived cell line PNT1A by FACS analysis. After treatment with EMTDTC-55 and EMTDTC-56, both floating and attached cells were pooled and double-stained with anti-Annexin V-APC and Calcein-AM. Cells that fluoresced in the green channel and stained positive for Calcien-AM were characterized as viable cells. Cells that fluoresced red only for APC stain were labeled as late apoptotic cells, while cells that fluoresced both green and red were labeled as early apoptotic cells. Cells that did not fluoresce after the double staining procedure were labelled as necrotic cells/debris.

Little or no drug effect occurred on day 1 in any of the cell lines as compared to the untreated controls. As shown in

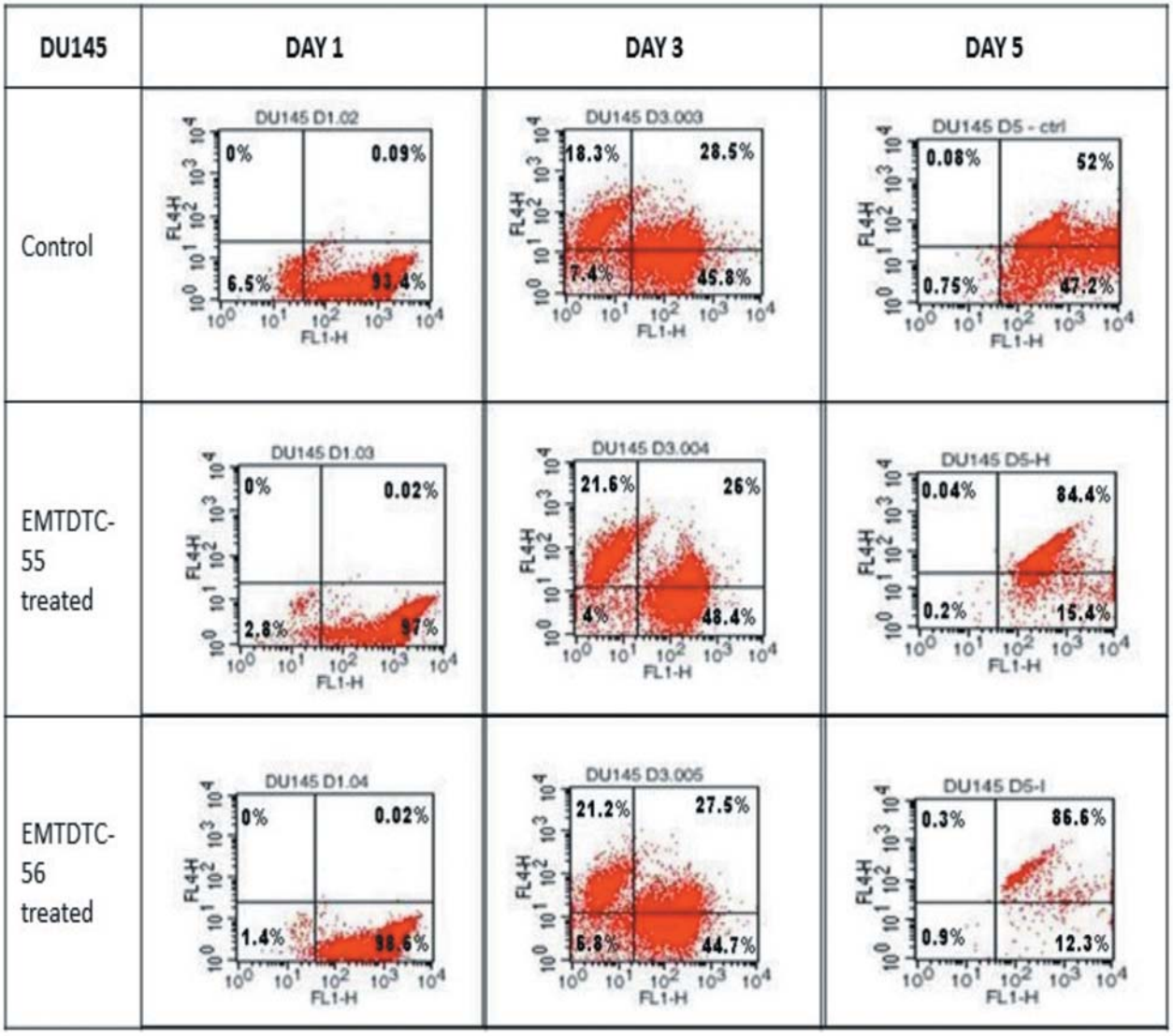

Figure 2. *Apoptosis in DU145 cells after treatment with EMTDTCs. Flow cytometry was performed on DU145 cells treated with EMTDTCs on Day 1, 3 and 5. The FL1-H (X-axis) is the green channel and the FL4-H (Y-axis) is the red channel by FACS analysis. Quadrants denote live (lower right), early apoptotic (upper right), late apoptotic (upper left) and necrotic (lower left) cells.*

Figure 1, on day 3, the apoptotic effect of EMTDTC-55 on PNT1A showed approximately 57% early apoptotic cells, less than 1% late apoptotic, while about 42% were viable. By day 5, EMTDTC-55 shifted the early apoptotic population of cells towards late apoptosis (15% were in late apoptosis and 42% still in early apoptosis), while maintaining a viable cell population at 41%. EMTDTC-56 exhibited a greater apoptotic potential than EMTDYC-55 as it showed 68% of cells in early apoptosis by day 3 and only less than 1% in late apoptosis. By day 5, compound EMTDTC-56 had induced 61% of cells into early apoptosis, ~9% in late apoptosis and 28% cells were viable.

As shown in Figure 2, in DU145 cells on day 1, the compounds produced no significant effects (97% cells were viable in the EMTDTC-55-treated cell group and 98% were viable in EMTDTC-56-treated cells). By day 3, both compounds showed increases in early and late apoptotic cell population. EMTDTC-55 data demonstrated 26% of cells in early apoptosis, ~22% in late apoptosis and 48% viable; however, by day 5, only 15% cells were viable and 84% in early apoptosis with no demarked late apoptotic cells. Compound EMTDTC-56 had a similar apoptotic potential on DU145, where ~27% of cells were in early apoptosis, 21% in late apoptosis and ~45% of cells were viable. By day 5, only

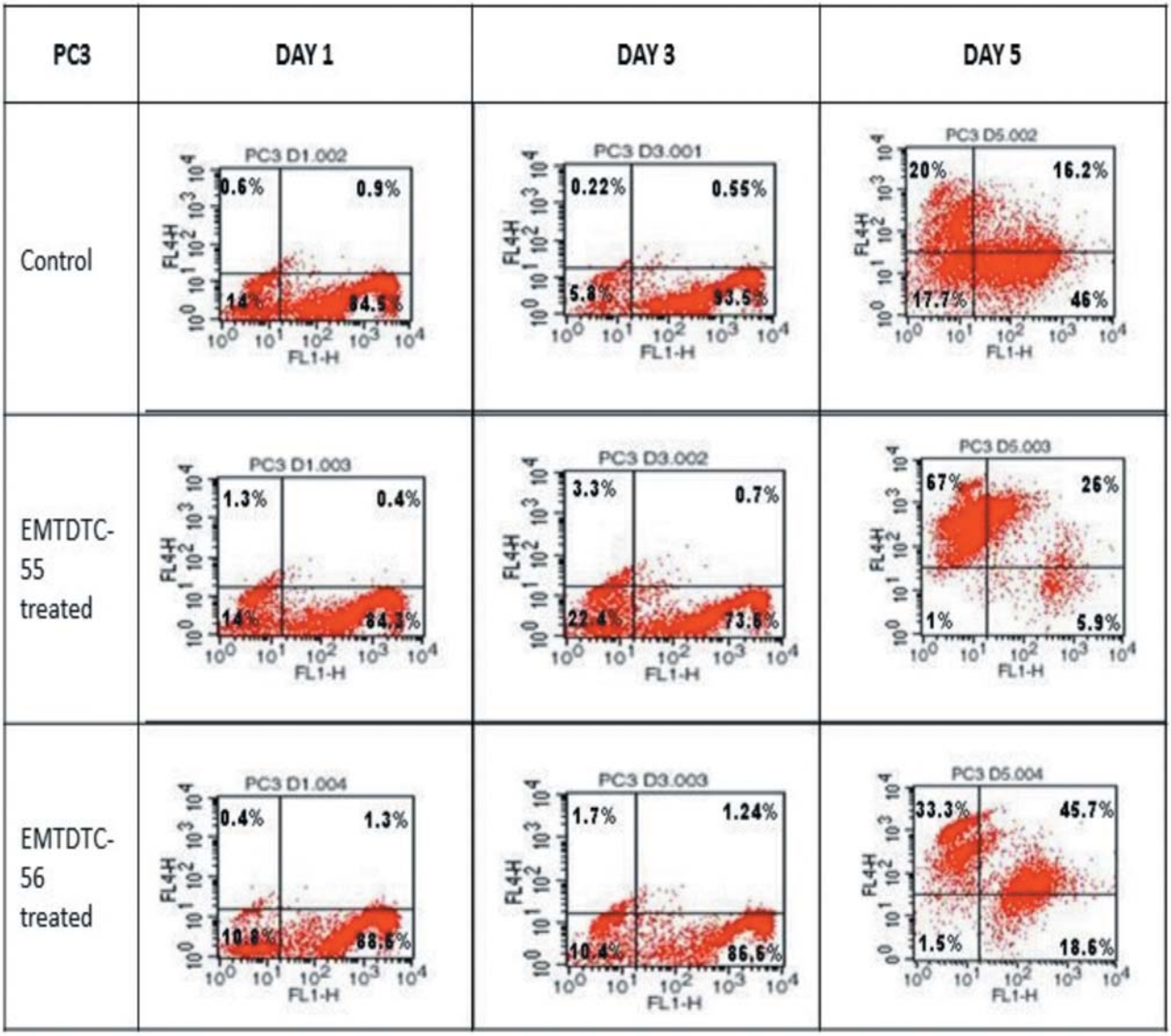

Figure 3. *Apoptosis in PC3 cells after treatment with EMTDTCs. Flow cytometry was performed on PC3 cells treated with EMTDTCs on Day 1, 3 and 5. The FL1-H (X-axis) is the green channel and the FL4-H (Y-axis) is the red channel by FACS analysis. Quadrants denote live (lower right), early apoptotic (upper right), late apoptotic (upper left) and necrotic (lower left) cells.*

12% of the cells were viable and ~87% in early apoptosis. We postulate that, by day 5, the apoptotic effects of EMTDTC-55 and EMTDTC-56 lead late apoptotic cells to be completely destroyed leaving no signs of necrotic cells or debris as the nuclear and cytoplasmic contents have completely leaked out.

As shown in Figure 3, in PC3 cells, compounds EMTDTC-55 and EMTDTC-56 showed ~74% and 86% of viable cells, respectively. On day 5, the control population of PC3 cells showed only ~46% of viable cells possibly due to nutrient media depletion, while 16% of cells were in early and 20% undergoing late apoptosis. Drawing a parallel comparison to the apoptotic potential of compound EMTDTC-55, 26% of cells were in early apoptosis, 67% in late apoptosis and only 5% of cells were viable. Compound EMTDTC-56 was relatively weaker than EMTDTC-55 at this experimental IC_{50} dose, whereby ~46% of cells were undergoing early apoptosis, 33% were in late apoptosis and ~19% were viable.

As shown in Figure 4, on day 1 in LNCaP cells, the data suggested that the apoptotic effect of both compounds sustained viable cell population numbers greater than 90%. By day 5, EMTDTC-55 suggested only 9% viable population, ~88% early

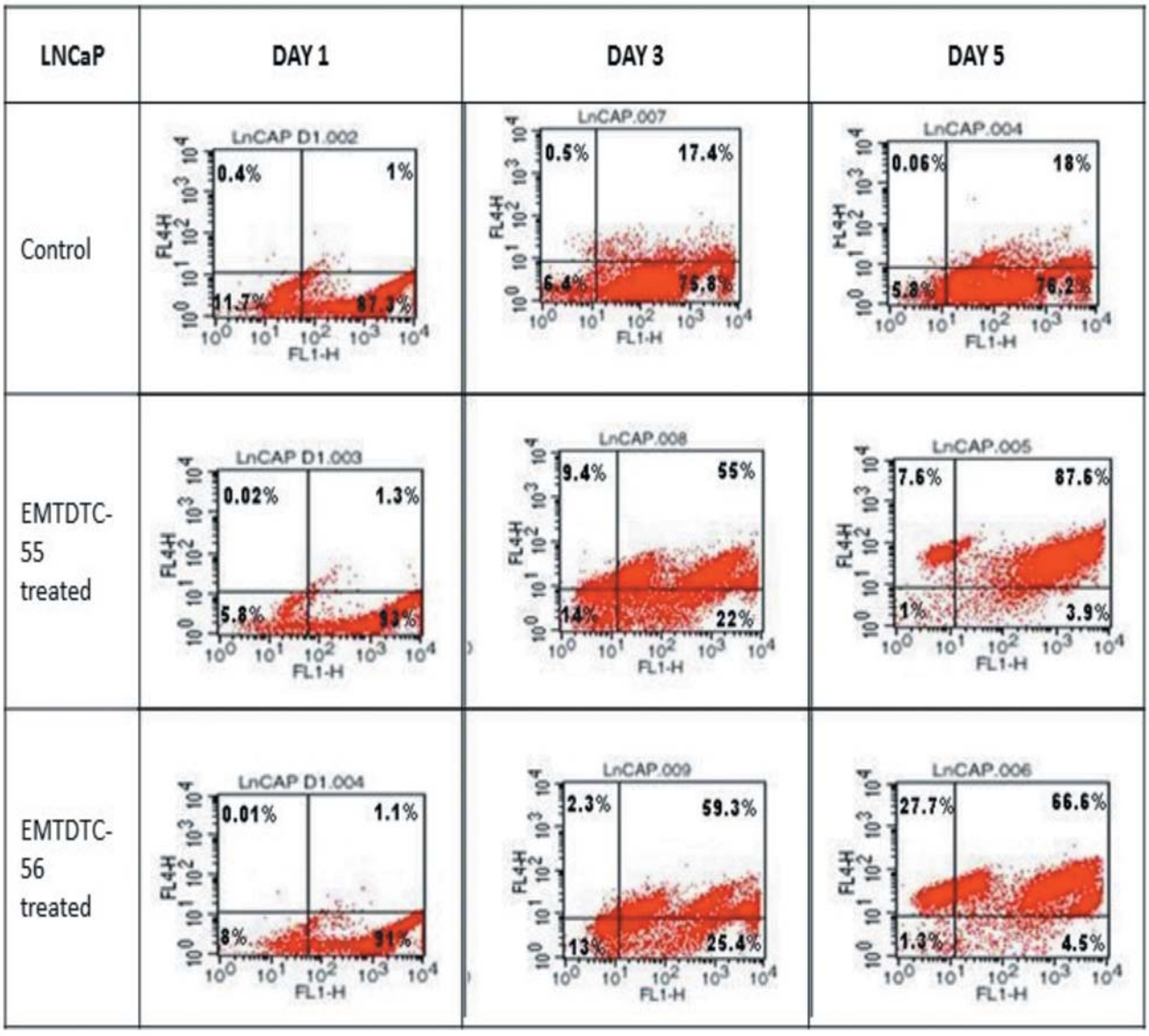

Figure 4. *Apoptosis in LNCaP cells after treatment with EMTDTCs. Flow cytometry was performed on LNCaP cells treated with EMTDTCs on Day 1, 3 and 5. The FL1-H (X-axis) is the green channel and the FL4-H (Y-axis) is the red channel by FACS analysis. Quadrants denote live (lower right), early apoptotic (upper right), late apoptotic (upper left) and necrotic (lower left) cells.*

apoptotic, 8% late apoptotic and 1% necrotic cells. EMTDTC-56 data showed a similar pattern where 5% of cells were viable, ~67% in early apoptotic, ~28% in late apoptotic and 1% in necrotic/debris state. Figure 5 summarizes the percentage of live cells after treatment with EMTDTCs in the four cell lines PNT1A, DU145, PC3 and LNCaP after treatment with EMTDTC-55 and EMTDTC-56 on days 1, 3 and 5.

EMTDTC analogs mediate regulation of key apoptotic genes. Genes that code for pro- and anti-apoptotic regulators were studied using mRNA expression profiles in cells treated with EMTDTCs. Fold changes greater than 4-fold are highlighted as significant in Table and plotted in Figure 6 and Figure 7.

Both compounds EMTDTC-55 and EMTDTC-56 mediated reduced expression of *TNF* in normal derivatized prostatic PNT1A cells. EMTDTC-55 reduced expression of *TNF* in androgen-independent cells DU145 with concurrent increase in expression of *NFKB1* and *PMEPA1* (~4-fold), *CDKN1A* (~5-fold) and VEGFA (~39-fold). Highly metastatic androgen-independent PC3 cells treated with

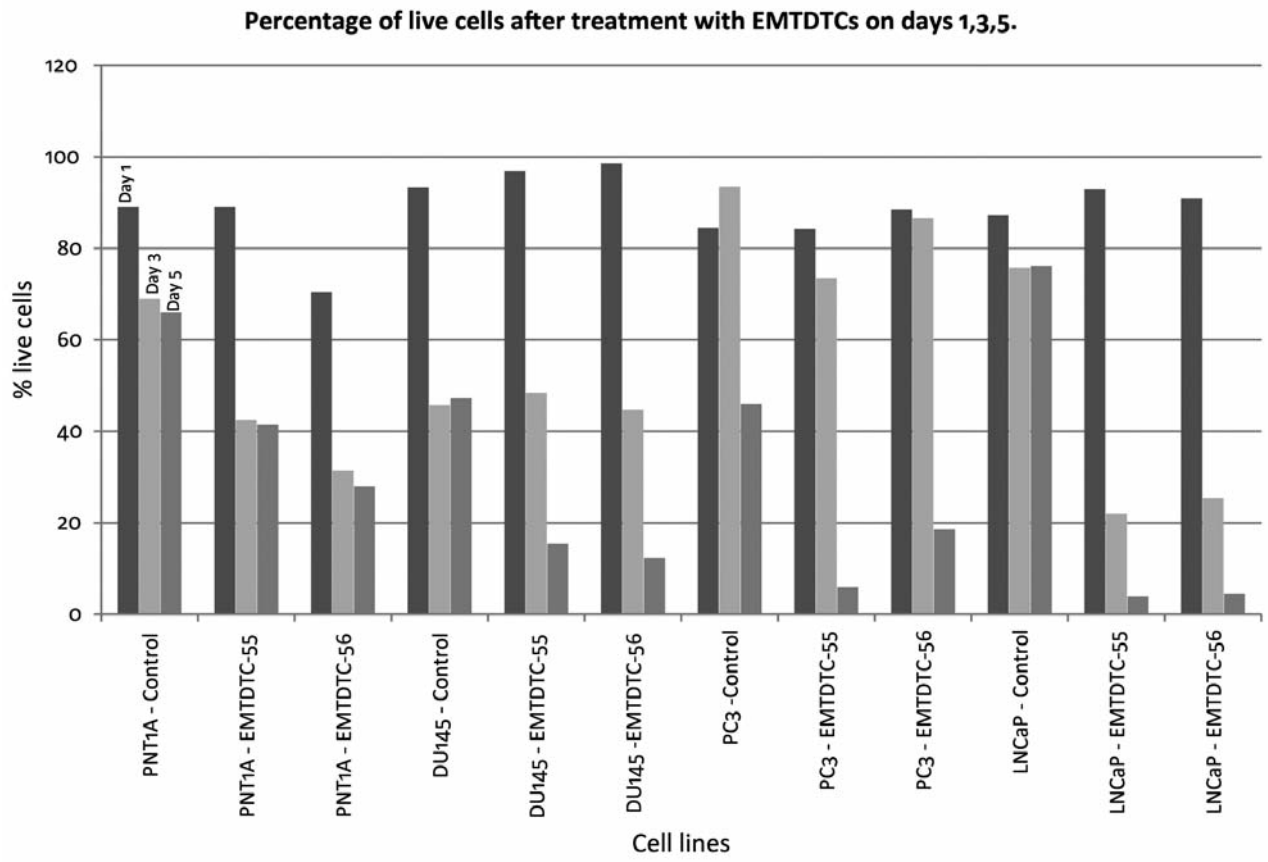

Figure 5. *Percentage of live cells after treatment with EMTDTCs. Cell lines PNT1A, DU145, PC3 and LNCaP were treated with EMTDTCs on days 1, 3 and 5.*

EMTDTC-55 had a ~20-fold increase in *PMEPA1*, which is involved in down-regulation of the androgen receptor, and increased ubiquitination and proteosomal degradation of the androgen receptor, along with an increased expression of *CDKN1A* (~9-fold), *NFKB1* (~7-fold) and TP53(~4-fold), while it reduced expression of *FASLG* (~330-fold). No notable changes were observed for apoptotic genes in LNCaP cells treated with EMTDTC-55.

PNT1A cells treated with EMTDTC-56 had a reduced expression of *TNF* and an increased impression of *FASLG*. In DU145 cells treated with EMTDTC-56, an increased expression of *VEGFA* (~48-fold) and *PMEPA1* (~15-fold) expression was observed, and a reduced expression of *TNF* (~30-fold). In PC3 cells treated with EMTDTC-56, an increased expression was observed in *TP53* (~330-fold), *PMEPA1* (~51-fold), *TNF* (~14-fold), *BCL2* (~12-fold), *CDKN1A* (~7-fold), *VEGFA* (~6-fold) and *NFKB1* (~5-fold), while *FASLG* showed a ~4-fold reduction. In contrast, in androgen-dependent LNCaP cells, we witnessed decreased expression in *TP53* (~48-fold), *TNF* (~20-fold), *CDKN1A* (~7-fold) and *PMEPA1* (~4-fold), while *VEGFA* showed a 4-fold increase.

Discussion

This study intended to identify key compounds from the group of derivatized dithiocarbamate analogs of emetine that have significant anti-apoptotic potential. The dithiocarbamate moiety attached to N2' position has been associated with anti-carcinogenic activities as previously mentioned (20). It was hypothesized that this moiety would provide reduced toxicity of emetine compounds whose extreme toxicity to various tissues limited its ability to function as a potential pharmacologic treatment in cancers.

The overall toxicity was assessed across the prostate cancer cell lines DU145, PC3 and LNCaP and compared to the normal derived PNT1A cell line. A MTT assay was performed at 5 concentrations of the compound EMTDTCs (data not shown).

The apoptotic rate was analyzed for the two compounds EMTDTC-55 and EMTDTC-56 by cell flow cytometry. EMTDTC-55 and EMTDTC-56 exhibited a comparable rates of apoptosis, however EMTDTC-56 lead to apoptosis at a faster rate than EMTDTC-55 across all cell types. In DU145

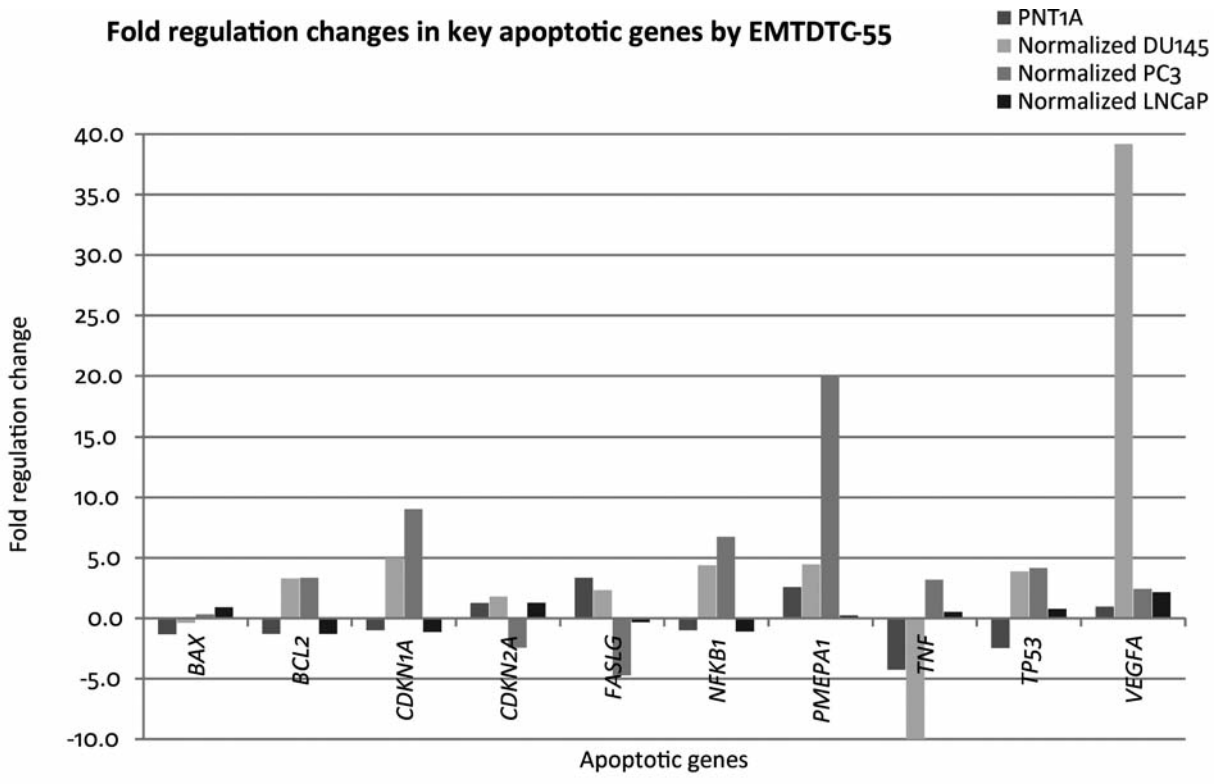

Figure 6. *Gene expression profiles of key apoptotic genes in cell lines PNT1A, DU145, PC3 and LNCaP upon treatment with EMTDTC-55.*

cells, by day 3, both compounds induced significant early apoptosis and by day 5 large populations of late apoptotic or necrotic cellular debris were disrupted, leaving no trace of late apoptotic cells for analysis. , Therefore, our results suggested the presence of a larger population of early apoptotic cells. The other plausible explanation could also be nutrient media deficit or toxicity that developed due to high cell death rate on day 3. In PC3 cells, the effects of EMTDTC-55 and EMTDTC-56 were not notably significant on day 3 (*i.e.* greater than 10% of cells in early apoptosis). However, by day 5, the PC3 cells were overcome by the anti-tumorigenic activity of EMTDTC-55, pushing 26% to early apoptosis and 67% to late apoptosis; only 5% were viable. Comparatively, EMTDTC-56-treated PC3 cells showed 46% of early apoptosis and 33% of late apoptosis, while ~19% remained viable on the same day. The flow cytometry data on LNCaP suggested little effect of both compounds on day 1, greater than 50% on day 3, while on day 5 more than 90% of cells were in early and late apoptosis additively.

Analysis of gene expression changes of key apoptotic genes in cell lines treated with EMTDTCs showed a distinct trend in androgen-independent and -dependent cell types. In normal derivatized prostate PNT1A cells, both EMTDTC-55 and EMTDTC-56 showed a decreased expression of *TNF* suggestive of decreased inflammatory and apoptotic signaling, counteractive to when TNF expression increase was inducing apoptosis. The ~40-fold increase in *VEGFA* in DU145 cells treated with EMDTC-55 is suggestive of signaling induced by hypoxia. EMTDTC-55 triggered a notable increase in *NFKB1* expression in androgen-independent DU145 and PC3 cells, which may indicate a feedback loop attempting to support pro-survival genes. *PMEPA1*, known for cell growth inhibition by the interaction of NEDD4 protein shunted for ubiquitin-proteasome pathway degradation (22), showed increased expression by ~4-fold in DU145 and ~20-fold in PC3 cells treated with EMTDTC-55. *CDKN1A* showed increased expression in DU145 and PC3 cells treated with EMTDTC-55; it is a cyclin-dependent kinase inhibitor that is tightly regulated by P53, which binds and inhibits cyclins CDK2 and CDK4, and function as a regulator of cell cycle progression past the G1 phase. Increased expression of *CDKN1A* could suggest cell cycle arrest and induction of apoptosis following caspase activation. EMTDTC-55 is a potent chemotherapeutic agent by apoptotic regulators in androgen-independent cell lines DU145 and PC3; no apoptotic genes were differentially regulated in androgen-dependent LNCaP cells.

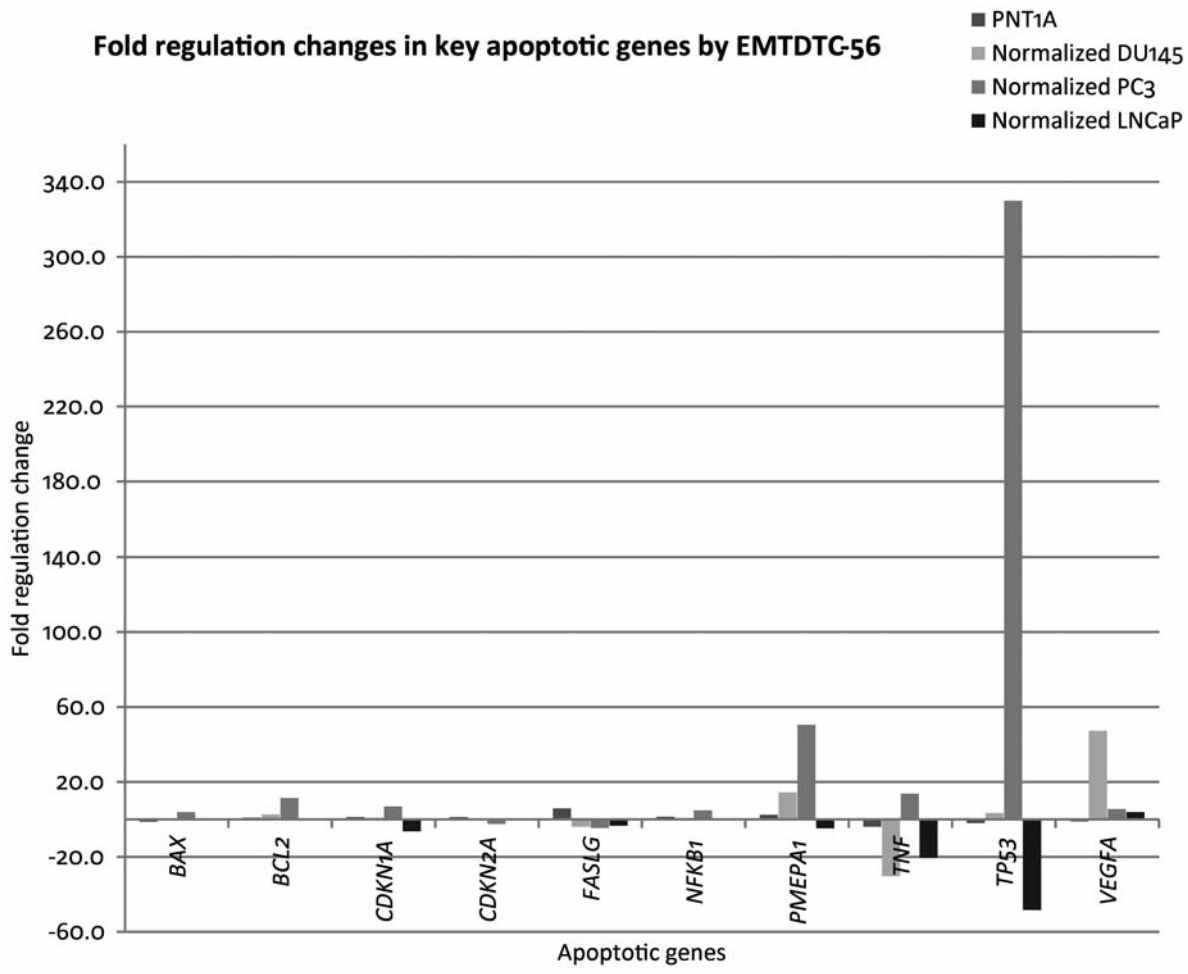

Figure 7. *Gene expression profiles of key apoptotic genes in cell lines PNT1A, DU145, PC3 and LNCaP upon treatment with EMTDTC-56.*

EMTDTC-56 was more efficacious in its differential regulation of apoptotic genes in DU145, PC3 and LNCaP. *VEGFA* was increased ~48-fold in DU145 and increased greater than 4-fold in PC3 and LNCaP cells, indicative of higher hypoxic conditions stimulated by increased cytotoxicity and selectivity in prostate cancer cell lines than normal PNT1A cells. EMTDTC-56 also showed a similar expression profile as EMTDTC-55 in DU145 with decrease in *TNF* and increase in *PMEPA1* gene regulation. In PC3 cells, large-fold changes of increased expression were observed for *PMEPA1* (~51-fold) and *TP53* (~330-fold) along with significant cell cycle arrest and apoptosis as indicated through flow cytometry. The notable role of EMTDTC-56 on *TP53* gene by 330-fold up-regulation is critical in its ability to act as a tumor suppressor in metastatic androgen-independent PC3 cells. Therefore, EMTDTC-56 has excellent potential as a chemotherapeutic drug against PC3 cells. The effects of EMTDTC-56 on apoptotic gene regulation in androgen-dependent LNCaP cells contrasted the profile of PC3. The apoptotic potential of EMTDTC-56 was noted through cytotoxicity data of MTT and apoptosis data of flow cytometry. Reduced expression of *TP53, TNF, PMEPA1* and *CDKN1A* in LNCaP cells treated with EMTDTC-56 suggests an alternative pathway of apoptosis induction in androgen-dependent cells.

Both emetine dithiocarbamate analogs showed significant chemotherapeutic potential in androgen-independent cell lines DU145 and PC3, while, although the data is significant for apoptosis in LNCaP, the alternate pathway has yet to be unraveled.

Conclusion

Novel dithiocarbamate analogs of emetine were synthesized and EMTDTC-55 and EMTDTC-56 showed potent anti-tumorigenic activity with minimal toxicity to normal prostate tissues and specific targeting of apoptotic regulatory genes.

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References

1 Grollman AP: Structural Basis for Inhibition of Protein Synthesis by Emetine and Cycloheximide Based on an Analogy between Ipecac Alkaloids and Glutarimide Antibiotics. Proc Natl Acad Sci USA *56(6)*: 1867-1874, 1966.

2 Lewisohn R: Action of emetin on malignant tumors JAMA *70*: 9-10, 1918.

3 Moller M, Herzer K, Wenger T, Herr I and Wink M: The alkaloid emetine as a promising agent for the induction and enhancement of drug-induced apoptosis in leukemia cells. Oncol Rep *18(3)*: 737-744, 2007.

4 Panettiere F and Coltman CA Jr: Experience with emetine hydrochloride (NSC 33669) as an antitumor agent. Cancer *27(4)*: 835-841, 1971.

5 Mastrangelo MJ, Grage TB, Bellet RE and Weiss AJ: A phase I study of emetine hydrochloride (NSC 33669) in solid tumors. Cancer *31(5)*: 1170-1175, 1973.

6 Siddiqui S, Firat D and Olshin S: Phase II study of emetine (NSC-33669) in the treatment of solid tumors. Cancer Chemother Rep *57(4)*: 423-428, 1973.

7 Moertel CG, Schutt AJ, Hahn RG and Reitemeier RJ: Treatment of advanced gastrointestinal cancer with ememtine (NSC-33669). Cancer Chemother Rep *58(2)*: 229-232, 1974.

8 Kane RC, Cohen MH, Broder LE, Bull MI, Creaven PJ and Fossieck BE Jr.: Phase I-II evaluation of emetine (NSC-33669) in the treatment of epidermoid bronchogenic carcinoma. Cancer Chemother Rep *59(6)*: 1171-1172, 1975.

9 Grollman AP and Huang MT: Inhibitors of protein synthesis in eukaryotes: tools in cell research. Fed Proc *32(6)*: 1673-1678, 1973.

10 Bicknell GR, Snowden RT and Cohen GM: Formation of high molecular mass DNA fragments is a marker of apoptosis in the human leukaemic cell line, U937. J Cell Sci *107(Pt 9)*: 2483-2489, 1994.

11 Kochi SK and Collier RJ: DNA fragmentation and cytolysis in U937 cells treated with diphtheria toxin or other inhibitors of protein synthesis. Exp Cell Res *208(1)*: 296-302, 1993.

12 Watanabe N, Iwamoto T, Dickinson DA, Iles KE and Forman HJ: Activation of the mitochondrial caspase cascade in the absence of protein synthesis does not require c-Jun N-terminal kinase. Arch Biochem Biophys *405(2)*: 231-240, 2002.

13 Meijerman I, Blom WM, de Bont HJ, Mulder GJ and Nagelkerke JF: Induction of apoptosis and changes in nuclear G-actin are mediated by different pathways: the effect of inhibitors of protein and RNA synthesis in isolated rat hepatocytes. Toxicol Appl Pharmacol *156(1)*: 46-55, 1999.

14 Jimenez A, Carrasco L and Vazquez D: Enzymic and nonenzymic translocation by yeast polysomes. Site of action of a number of inhibitors. Biochemistry *16(21)*: 4727-4730, 1977.

15 Martin TF: Paradoxical effects of protein synthesis inhibitors on uridine uptake in cultured cells: possible role of uncharged tRNA in regulating metabolism. J Cell Physiol *103(3)*: 489-502, 1980.

16 Chitnis MP and Johnson RK: Biochemical parameters of resistance of an adriamycin-resistant subline of P388 leukemia to emetine, an inhibitor of protein synthesis. J Natl Cancer Inst *60(5)*: 1049-54, 1978.

17 Hwang KM, Yang LC, Carrico CK, Schulz RA, Schenkman JB and Sartorelli AC: Production of membrane whorls in rat liver by some inhibitors of protein synthesis. J Cell Biol *62*: 20-31, 1974.

18 Lietman PS: Mitochondrial protein synthesis: inhibition by emetine hydrochloride. Mol Pharmacol *7(2)*: 122-128, 1971.

19 Klatskin G and Friedman H: Emetine toxicity in man; studies on the nature of early toxic manifestations, their relation to the dose level, and their significance in determining safe dosage. Ann Intern Med *28(5)*: 892-915, 1948.

20 Akinboye ES, Rosen MD, Denmeade SR, Kwabi-Addo B and Bakare O: Design, Synthesis, and Evaluation of pH-Dependent Hydrolyzable Emetine Analogues as Treatment for Prostate Cancer. Journal of medicinal chemistry *55(17)*: 7450-7259, 2012.

21 Akinboye ES, Bamji ZD, Kwabi-Addo B, Ejeh D, Copeland Jr RL, Denmeade SR and Bakare O: Design, Synthesis and Cytotoxicity studies of dithiocarbamate ester derivatives of emetine in prostate cancer cell lines. Bioorganic & Medicinal Chemistry. In press, 2015. DOI: 10.1016/j.bmc.2015.06.072

22 Xu LL, Shi Y, Petrovics G, Sun C, Makarem M, Zhang W, Sesterhenn IA, McLeod DG, Sun L and Moul JW: PMEPA1, an androgen-regulated NEDD4-binding protein, exhibits cell growth inhibitory function and decreased expression during prostate cancer progression. Cancer Res *63(15)*: 4299-4304, 2003.

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