Anti-tumor Effect of Novel Cationic Biomaterials in Prostate Cancer

GUNJAN GAKHAR[2*], HUIXIAN LIU[1*], ROQUIAN SHEN[2], DOUGLAS SCHERR[1], DEQUN WU[3], DAVID NANUS[2] and CHIH-CHANG CHU[3]

Departments of [1]Urology and [2]Medicine, Weill Cornell Medical College, New York City, NY, U.S.A.; [3]Biomedical Engineering Program and Department of Fiber Science and Apparel Design, Cornell University, Ithaca, NY, U.S.A.

Abstract. *Background: Tumor cells expressing excessive anionic-charged sialic acid can be potentially targeted by cationic polymers which may inhibit tumor growth. In the present study, three new families of cationic polymers were synthesized to assess their effects on prostate cancer cells. Materials and Methods: Cationic polymers effects on PC3 prostate cancer cells and normal prostate epithelial cell (RWPE-1) were assessed using cell viability, DNA fragmentation, apoptosis assays and confocal microscopy. Results: The dextran-based polymer (Dex-PA-3X) (40 μg/ml) and the vinyl-based PolyAETA (5 μg/ml) induced a significant reduction in cell viability in PC3 cells (85% and 50%, respectively; p<0.05) in comparison to RWPE-1 cells. Furthermore, Dex-PA-3X induced a 50%, and PolyAETA induced a 35% increase in cell death in PC3 cells compared to RWPE-1 cells measured by DNA fragmentation assay. Lower concentrations of both polymers induced apoptosis while higher concentrations induced both apoptosis and necrosis by immunostaining. Confocal microscopy indicated the localization of Dex-PA in the cytoplasm of PC3 but not RWPE-1 cells, while PolyAETA was seen in both PC3 and RWPE-1 cells, but at lower intensity in RWPE-1 cells. Conclusion: The newly-synthesized cationic polymers Dex-PA-3X and PolyAETA selectively bind to, reduce viability and induce cell apoptosis in prostate cancer cells, suggesting that targeting negatively-charged tumor cells could be a novel strategy to treat prostate cancer.*

*These Authors contributed equally to this study.

Correspondence to: C.C. Chu, Biomedical Engineering Program and Department of Fiber Science and Apparel Design, Cornell University, Ithaca, NY, 14853-4401, U.S.A. Tel: +1 6072551938, Fax: +1 6072551093, e-mail: cc62@cornell.edu

Key Words: Amino acid-based poly (ester-amide)s, polysaccharides, dextran, chitosan, cationic polymers, prostate cancer, cytotoxicity, apoptosis, necrosis, arginine, lysine.

Several studies suggest that malignant cells are more negatively-charged compared with normal cells due to the overexpression of sialic acid on their cell surface (1-4). Tumor cells expressing polysialic acid tend to dissociate from each other due to electrostatic repulsion leading to cell migration into the circulation (5-7), which may contribute to increased invasiveness and metastatic potential (8-10). Sialic acid has a dissociation constant of 2.6, an indication of a strong negative charge at pH 7.4. Consequently, the negatively-charged cell surface attributed to sialic acid on malignant cells may represent a potential drug target. Holmberg *et al*. reported that the growth of tumor cell lines including prostate cancer cells was inhibited by positively-charged cationic dextran derivatives (11) in a concentration-dependent fashion, suggesting that positively charged polymers represent a novel strategy to target and inhibit the growth of malignant cells.

A variety of novel positively- and negatively-charged water soluble polymers have recently been synthesized, including polymers derived from natural polysaccharides like chitosan and dextran, from synthetic original like amino acid-based poly(ester amide)s (AA-PEA) and a new class of water soluble cationic polymer (PolyAETA) synthesized from the [2-(acryloxy)ethyl]trimethylammonium chloride] vinyl-based monomer (AETA) (12-25). Many of these polymers have been tested as non-viral gene vectors for transfecting cells (23, 25-27) and scaffolds for supporting cell proliferation (20, 28), while being safely tested in humans for a wide range of biomedical applications ranging from coating for drug-eluting stents (29, 30), synthetic vaccines (31) and drug-eluting fibrous membranes as dressing for treating burn wounds (32).

In the current study, we screened multiple polymers from three families of cationic natural and synthetic polymers (dextran and chitosan from the polysaccharide family, Arg- and Lys-based PEAs from the AA-PEA family and PolyAETA from the vinyl family) for their ability to inhibit growth of prostate cancer cells when compared to the RWPE-1 control.

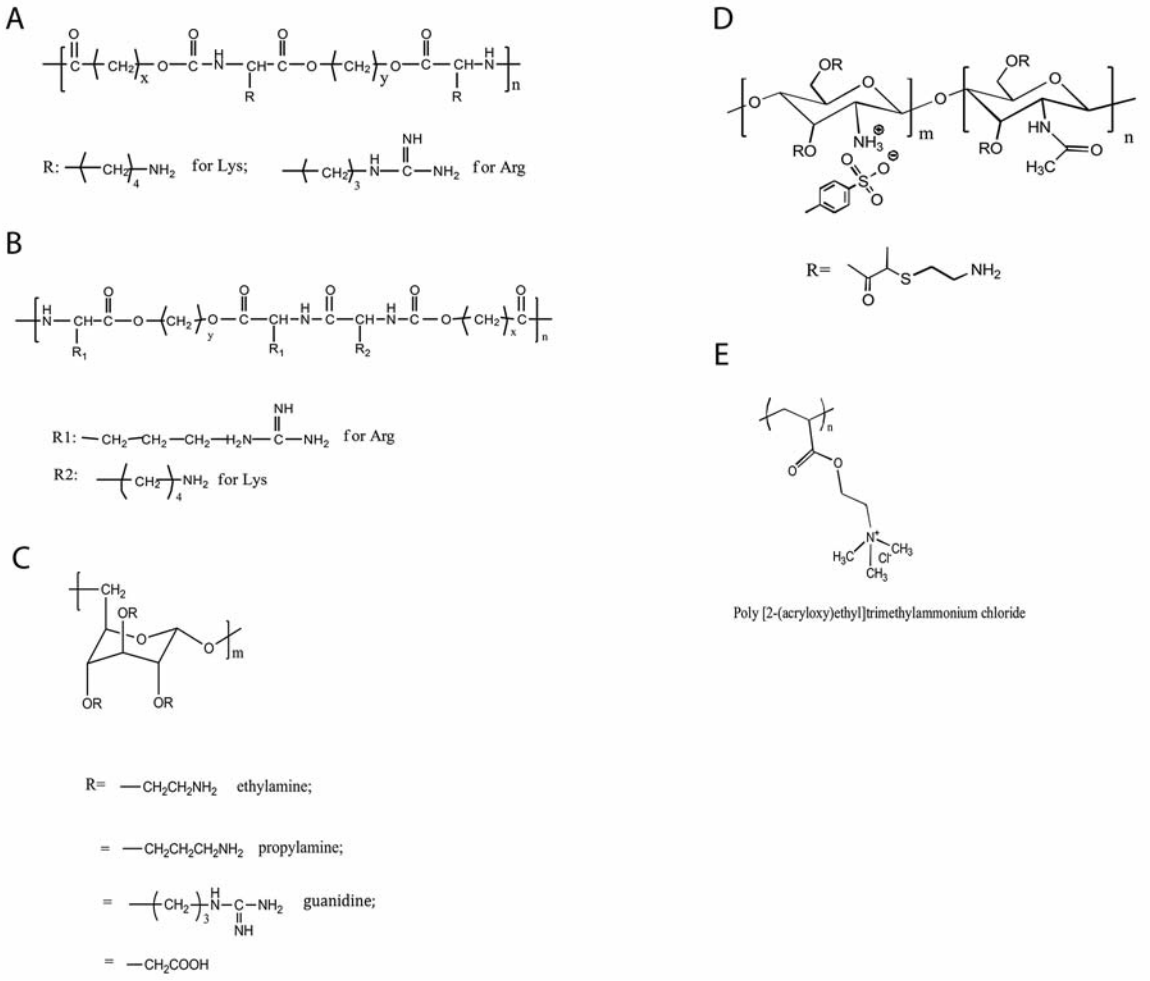

Figure 1. *Generic chemical structures of the polymers tested. A) Repeating unit structure of a cationic amino acid-based poly (ester amide)s with Arg or Lys as the amino acid building block. B) Multiamino acid-based poly (ester amide)s having both Arg and Lys within the same repeating unit of the AA-PEA polymer. C) Dextran-based cationic polymers and their anionic control. Depending on the R group, Dex-EA, Dex-PA, and Dex-GND are shown. The R –CH_2COOH (Dex-CA) was used as the anionic control for the dextran-based cationic polymers. D) GMA-chitosan-NH_2. Glycidyl methacrylate-chitosan (GMA-chitosan) as the parent polysaccharide and its vinyl end group in the GMA moiety is converted into thioamine to fabricate GMA-chitosan-NH_2. E) Synthetic cationic vinyl polymer, PolyAETA.*

Based on the polymer screening data, an in-depth study was performed on two selected candidates to further characterize their biological interactions with prostate cancer cells.

Materials and Methods

Structure of amino-acid based poly (ester) amide polymers, polysaccharide-based polymers and PolyAETA. The structure of the polymers, including chemical formulas are described in the Figure 1 and Table I.

Cell culture and reagents. Both human prostate cancer cell line PC3 and RWPE-1 (normal prostate epithelial cells) were purchased from American Type Culture Collection (Manassas, VA, USA) and maintained as previously described (33). RWPE-1 cells were grown in Roswell Park Memorial Institute medium (RPMI, Sigma-Aldrich, St. Louis, MO, USA) supplemented with 0.05 mg/ml bovine pituitary extract and 5 ng/ml epidermal growth factor while PC3 cells were kept in RPMI growth medium.

MTT assay. Cell proliferation and viability were measured using CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (MTT) (Promega, Madison, Wisconsin, USA) according to the manufacturer's instructions. Briefly, 10^4 cells in 100 μl RPMI medium were seeded in 96-well plates and cultured in 5% Fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, MO, USA) for 24 hours. The wells were washed once with PBS and 100 μl fresh medium was added with 1% FBS containing each of the polymers shown in the Supplementary Table at 100 μg/ml for 4 days and kept at 37˚C, 5% CO_2 incubator. On the day of testing, 15 μl of MTT labelling reagent (5 mg/ml MTT) was added to each well and the mixture was incubated for 2-4 h at 37˚C, then 100

μl of solubilizing reagent was added and the plate was incubated at room temperature (RT) for 2 h or overnight to dissolve formazan crystals. Absorbance was measured at OD_{595} in a Chameleon multilabel detection platform (Hidex Inc., Mustionkatu, Turku, Finland). Each assay was carried out in triplicate and each experiment was repeated at least twice.

DNA fragmentation ELISA assay. PC3 cells were cultured in media containing 5-bromo-2-deoxyuridine (BrdU) reagent (1:1000 from cellular DNA Fragmentation ELISA kit, Roche-applied-science, Tucson, AZ, USA) at 37˚C for 2-3 days without changing the medium. Labeled cells were trypsinized and aliquoted into a 96-well microplate at a concentration of 1×10^5 cells/ml in RPMI medium with 10% FBS, and washed once with PBS on the second day before adding 100 μl fresh RPMI medium supplemented with 1% FBS. Dex-PA-3X (10, 40 and 160 μg/ml) and PolyAETA (2.5, 5 and 10 μg/ml) were individually added into the RPMI medium for 24 h, the microplate centrifuged for 5 min and the supernatants added to a fresh testing microplate previously coated with anti-DNA antibody. The plate was placed on a shaker at 200 rpm for 90 min at RT, washed with washing buffer 3 times, microwaved for 4 min, cooled down at RT, placed at –20˚C for 10 min and then washed with washing buffer. Anti-BrdU-peroxidase was then added and incubated at RT for 90 min on a rotator. The plate was washed 3 times and 100 μl substrate solution added and absorbance was measured at wavelength 370 nm with the reference wavelength of 492 nm. Each assay was carried out in triplicate and each experiment was repeated at least twice.

Propidium iodide (PI) staining. To detect necrosis induced by polymers, PC3 cells were seeded on the coverslips in a 12-well plate (Thermo Fisher Scientific, Waltham, MA, USA), and cultured in RPMI medium with 10% FBS overnight, washed with PBS and incubated in media containing PolyAETA at 5 and 20 μg/ml or Dex-PA-3X at 40 and 160 μg/ml for 24 h. Following incubation, cells were washed with PBS, stained with 10 μg/ml Propidium iodide (PI; Invitrogen, Grand Island, NY, USA) for10 min at RT, washed with PBS and fixed with 4% paraformaldehyde for 20 min at RT. After fixation, cells were rinsed twice with distilled water and stained with 0.5 μg/ml DAPI (4', 6-Diamidino-2-Phenylindole, Dilactate, Sigma-Aldrich, St. Louis, MO, USA) for 2 min. After washing with distilled water, slides were dried and mounted for fluorescence microscopy. PI-negative were considered live and apoptotic cells and PI-positive were considered necrotic and "apoptonecrotic" cells.

Cleaved caspase-3 immunofluorescence staining. PC3 cells were seeded on coverslips in a 12-well plate and incubated with media containing Dex-PA-3X (40 and 160 μg/ml) and PolyAETA (5 and 20 μg/ml) for 24 h and cells were fixed with 4% formaldehyde for 15 min at RT, rinsed three times in PBS for 5 min each, incubated in blocking buffer (4% BSA in PBS) for 60 min, washed and then incubated in cleaved caspase-3 (Asp175) antibody (Alexa Fluor® 488 Conjugate; Cell Signaling Technology, Danvers, MA, USA) diluted 1:100 in the dilution buffer for 60 min at RT. Cells were rinsed three times in PBS for 5 min and the coverslips were mounted with Prolong® Gold Antifade Reagent (Thermo Fisher Scientific, Waltham, MA, USA) and observed under fluorescence microscopy immediately.

Immunofluorescence and confocal microscopy. To determine cellular localization of polymers, PolyAETA and Dex-PA-1X were fluorescently conjugated. Due to the consumption of all the available hydroxyl groups in the Dex-PA-3X, no free hydroxyl groups could be used to conjugate a fluorescence dye onto Dex-PA-3X. PC3 and RWPE-1 cells grown on coverslips in 48-well plates in RPMI media were incubated with 100 μg/ml fluorescently conjugated PolyAETA and Dex-PA-1X for 30 min, and 5 h, rinsed with PBS and fixed with 2% paraformaldehyde for 20 min. After washing with PBS, cells were blocked with 2.5% BSA in PBS for 1 h and then incubated with Alexa fluor 568 labeled mouse anti-EpCAM (BioLegend, San Diego, CA, USA) 1:100 overnight at 4˚C. Cells were washed with PBS, incubated in DAPI for 5 min, rinsed with distilled water and analyzed by a confocal microscope (Carl Zeiss LSM 510 META, Narashige, MN, USA) for localization of the polymers inside the cells.

Table I. *List of polymers*.

AA-PEA family (Net charge)	Polysaccharide family (Net charge)	Vinyl-based family (Net charge)
2A3S	Dex-EA	*PolyAETA
4A3S	*Dex-PA1x	PAA
4A3ES	Dex-PA3x	
8A7ES	Dex-GND	
2A4L-25	GMA-NH2	
4L4NH2	Dex-CA	

All the polymers are positively charged except Dex-CA and PAA (negatively charged) used as controls. *Polymers used for in-depth analysis.

Statistical analysis. The level of significance was considered at $p<0.05$ using Student's *t*-test analysis. All data are presented as mean±S.D. of at least three independent experiments from different batches of cultures. Figures are representative of one triplicate experiment.

Results

Effect of polymers on cell viability in prostate cells. For a rapid *in vitro* polymer screening of prostate cancer cells, PC3 cells were incubated with a fixed concentration (100 μg/ml) of different cationic and anionic polymers and cell viability was measured at 4 days using an MTT assay (Figure 2). Among the 12 cationic polymers tested, PolyAETA (vinyl-based) and Dex-PA (polysaccharide-based) showed a significant reduction in cell viability, with only <20% PC3 cells viable ($p<0.05$). AA-PEA polymers had little or no effect compared to the PBS control. Figure 1 provides the chemical structure of all tested polymers.

Dex-PA-3X and PolyAETA polymers were further analyzed at various concentrations on RWPE-1 normal prostate epithelial cells and PC3 cells. Dex-PA-3X showed an 85% and 88% decrease ($p<0.05$) in the cell viability in PC3 cells in comparison to RWPE-1 cells at 40 and 160 μg/ml, respectively (Figure 3A). Similarly, PolyAETA showed a significant decrease (45%, $p<0.05$) in cell viability in PC3 cells in comparison to RWPE-1 cells at 5 μg/ml (Figure 3B)

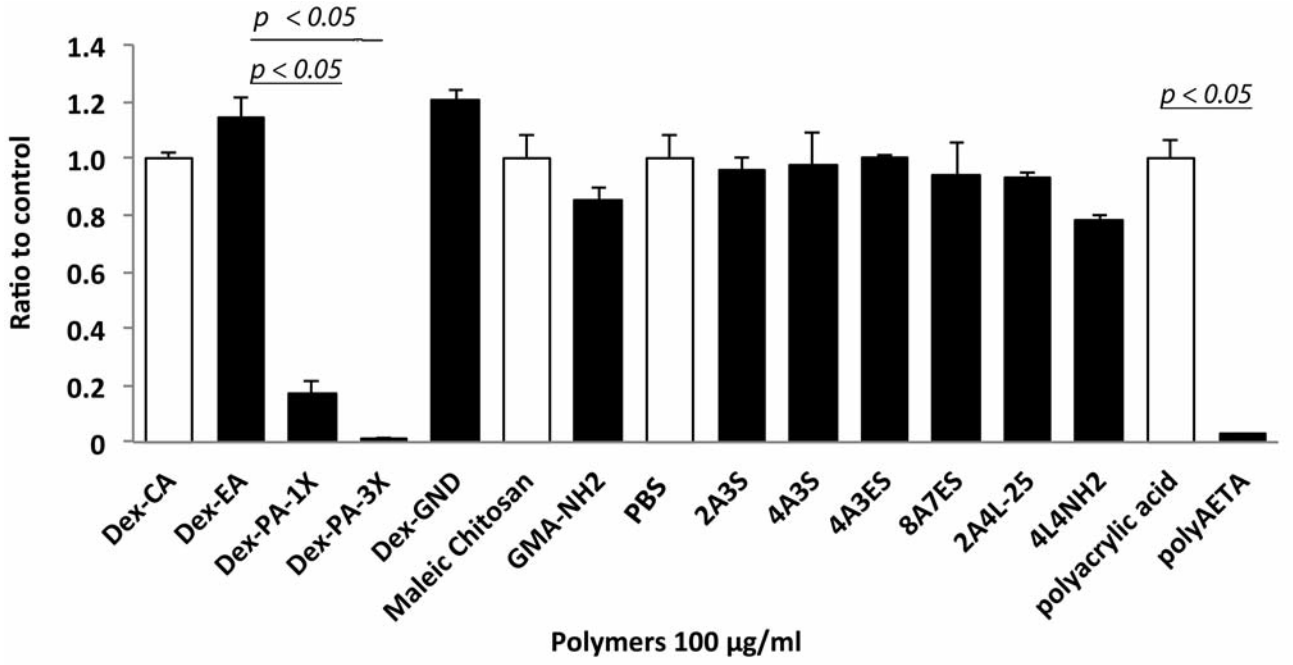

Figure 2. *MTT assay was performed to screen the effect of different polymers on PC3 cells. Refer to Table I for the testing polymers. Vertical axis indicates the ratio of viable cells of testing polymers to their corresponding controls that were set at the value 1. Therefore, the lower the bars, the more effective are the polymers in reducing cancer cell viability. PBS was used as the control for all AA-PEA-based cationic polymers since no water soluble anionic AA-PEAs could be synthesized. The plain white bars are controls for the respective polymers which are black bars next to them. After incubated with PC3 cells for 96 hours at the indicated concentration, Dex-PA-1X, Dex-PA-3X and PolyAETA showed a prominent effect in reducing viability of PC3 cells comparing to the other polymers. Both Dex-PA-1X and 3X belong to polysaccharide, while PolyAETA belongs to vinyl-based family.*

while at 20 μg/ml, both RWPE-1 and PC3 cells showed a similar reduction in cell viability. These data showed that both polymers inhibit PC-3 cells more than RWPE-1 cells.

Characterization of polymer-induced cell death in prostate cells. We next examined the effects of PolyAETA and Dex-PA-3X on inducing apoptosis. PC3 and RWPE-1 cells were treated with Dex-PA-3X for 24 h at 40 and 160 μg/ml concentrations (Figure 4A). Dex-PA-3X induced a 50% increase in apoptosis in PC3 cells compared with RWPE-1 cells at both 40 and 160 μg/ml ($p<0.05$), as determined by DNA fragmentation ELISA assay. PolyAETA at 5 and 20 μg/ml induced a 35% increase in apoptosis in PC3 cells in comparison with RWPE-1 cells (Figure 4B, $p<0.05$). To further explore the mechanism of PolyAETA and Dex-PA-3X induced cell death, PI and caspase-3 staining was performed on PC3 cells. As shown in Figure 5, cationic Dex-PA-3X at 40 μg/ml induced cell death in PC3 cells mainly by apoptosis while at 160 μg/ml cell death was induced both by necrosis and apoptosis (Figure 5A). Similarly, PolyAETA at 5 μg/ml induced cell death primarily by apoptosis, while at 20 μg/ml, both necrosis and apoptosis were observed. In comparison to Dex-PA-3X (160 μg/ml), PolyAETA (20 μg/ml) induced more necrosis in PC3 cells.

Localization of the polymers in RWPE-1 and PC3 cells. Confocal microscopy was performed to determine where polymers were localized in cells following binding to the cell surface. At 1 h, both Dex-PA-1X and PolyAETA were localized in the cytoplasm of PC3 cells and to a much lesser extent in RWPE-1 cells (Figure 6), supporting the hypothesis that the presence of negatively charged cell surface sialic acid on cancer cells permits a more robust interaction and internalization of cationic polymers in comparison to non-malignant cells. After 5 h, the amount of both PolyAETA and Dex-PA-1X polymers increased in the cytoplasm of PC3, but not RWPE-1 cells (Figure 6A and 6B).

Discussion

In the present study, a number of new cationic and water soluble polymers ranging from natural-based like dextran and chitosan to synthetic like poly(AETA) and AA-PEAs were synthesized to examine their effect on prostate cells. The cationic polymers were synthesized based on the hypothesis that tumor cells express higher amounts of sialic acid than normal cells generating a higher negative charge on their cell surfaces that would facilitate uptake of cationic compounds.

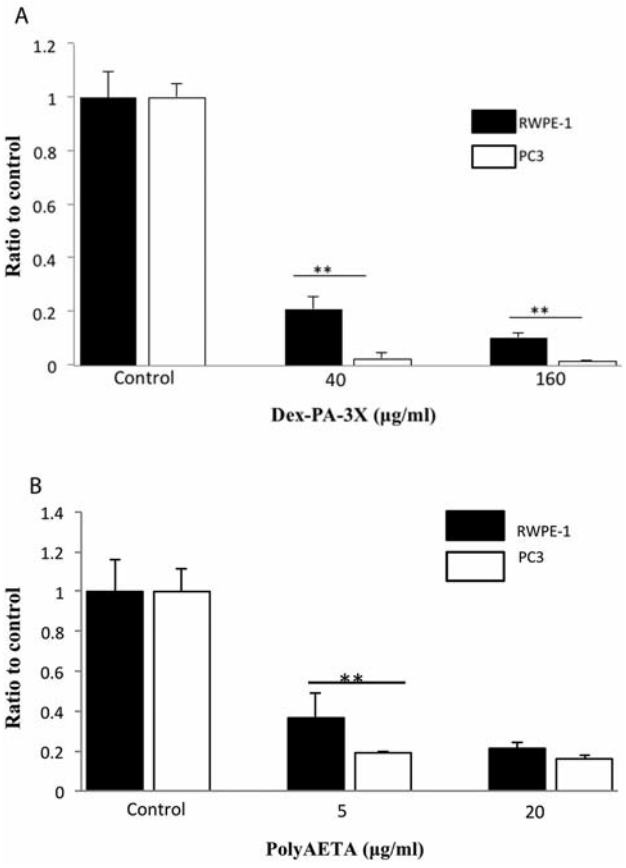

Figure 3. *Effect of Dex-PA-3X and Poly AETA on cell viability in PC3 and RWPE-1 cells. A) PC3 and RWPE-1 cells were treated with Dex-PA-3X for 24 h at indicated concentrations with Dex-CA as control. As shown, Dex-PA-3X is far more cytotoxic to cancerous PC3 cells than to normal prostate epithelial RWPE-1 cells at both concentrations of 40 and 160 µg/ml. A 85% and 88% more reduction in cell viability were induced in PC3 cells than in RWPE-1 cells by Dex-PA-3X at 40 µg/ml and 160 µg/ml, respectively. **p<0.05. B) PC3 and RWPE-1 cells were treated with PolyAETA for 24 h at indicated concentrations with polyacrylic acid as control. As shown, PolyAETA is significantly more cytotoxic to cancerous PC3 cells than to normal prostate epithelial RWPE-1 cells at the concentrations of 5µg/ml. About 45% more reduction in cell viability was induced in PC3 cells than in RWPE-1 cells by PolyAETA at 5 µg/ml. **p<0.05.*

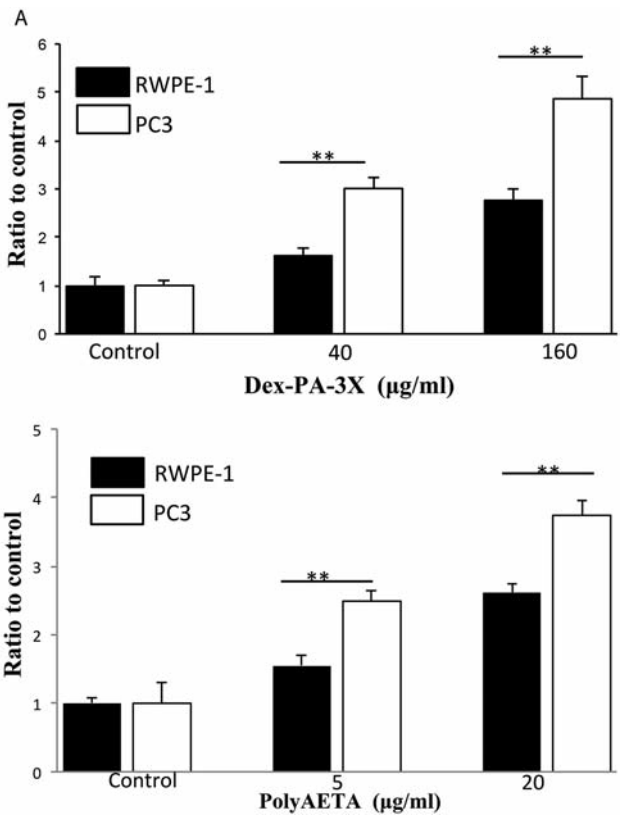

Figure 4. *Effect of Dex-PA-3X and PolyAETA on cell apoptosis in PC3 and RWPE-1 cells. A) PC3 and RWPE-1 cells were treated with Dex-PA-3X for 24 h at 40 and 160 µg/ml concentrations. A significant increase in cell death was seen in PC3 cells compared to RWPE-1 cells at all concentrations. **p<0.05. B) PC3 and RWPE-1 cells were treated with PolyAETA for 24 h at 5 and 20 µg/ml concentrations. A significant increase in cell death was seen in PC3 cells compared to RWPE-1 cells at all concentrations. **p<0.05. PC3 and RWPE-1 cells were treated with Dex-PA-3X for 24 h at 40 and 160 µg/ml concentrations. A significant increase in cell death was seen in PC3 cells compared to RWPE-1 cells at all concentrations. **p<0.05.*

Sialic acids are a group of neuraminic acid widely present as sugars on the terminal positions of glycoproteins or glycolipids. Overexpression of sialylated antigens has been commonly observed in several epithelial cancers such as gastric, pancreatic, breast, ovarian and colorectal, and usually linked with poor prognosis (34). The poor prognosis may be due to increased metastatic potential of tumors expressing sialic acid as sialyl-glycoconjugates regulate adhesion and promote motility, while inhibition of sialylation reduces the metastatic potential of cancer cells (35).

Among the 12 cationic polymers examined in the current study, Lys-PEAs, GMA-chitosan-NH_2, maleic chitosan (as the control to GMA-chitosan-NH_2), Dex-GND and Poly (AETA) are being reported for the first time, and the remaining were synthesized according to prior published studies (13, 14, 19, 23, 26, 27). In the present study, we tested the effect of cationic polymers in both cancerous and non-cancerous prostate cells, while prior published studies did not test the relative efficacy of cationic polymers in malignant versus non-malignant prostate cells (11, 36-38).

Based on our results, the cytotoxicity of cationic polymers can be ranked as follows; Dex-PA-3X> PolyAETA> Dex-PA-1X> 4L4NH2> GMA-NH2> 2A4L-25> 8A7ES> 2A3S> 4A3S> 4A3ES> Dex-EA> Dex-GND. Dex-PA-3X and

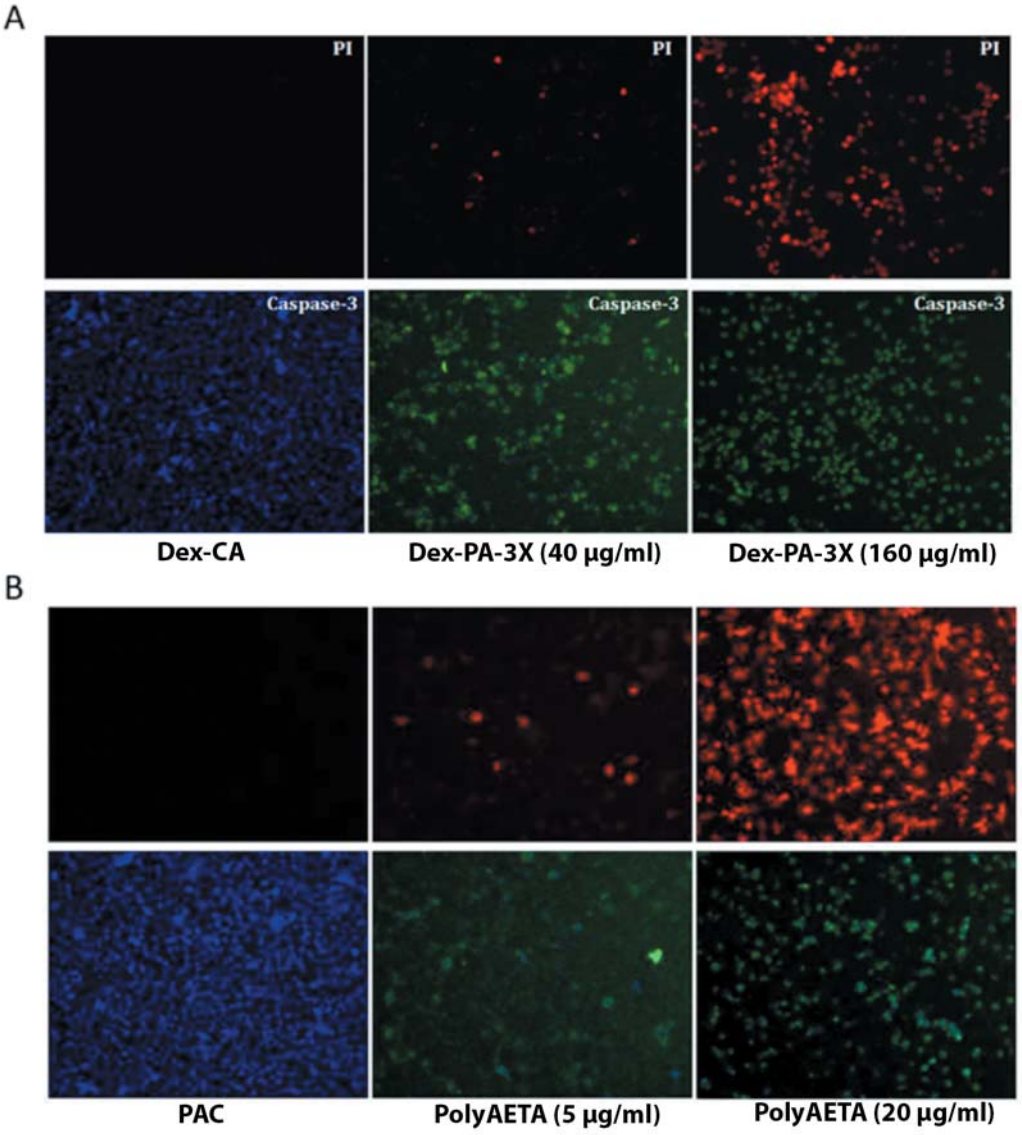

Figure 5. *Propidium iodide and cleaved caspase-3 staining for cell death induced by PolyAETA and Dex-PA-3X. Cells undergoing necrosis or necroptosis are stained red with propidium iodide (PI) and cells undergoing apoptosis are stained green with cleaved caspase-3 antibody. DAPI was used to stain the nuclei of all cells: A) PC3 cells treated with Dex-PA-3X for 24 h at indicated concentrations. B) PC3 cells treated with PolyAETA for 24 h at indicated concentrations. PAA and Dex-CA were used as controls for PolyAETA and Dex-PA-3X respectively.*

PolyAETA belonging to dextran-based and vinyl-based class of polymers, respectively, demonstrated the most significant cytotoxic effects.

Among polysaccharides, Dex-PA showed an increased cytotoxicity than Dex-EA which could be due to the: (i) presence of increased number of free amino end groups; (ii) longer spacer between the free amino end group and the dextran backbone; or (iii) hydrophobic nature of propylamine-pendant group allowing for better interaction with the cell membrane. The lack of cytotoxicity of the cationic Dex-GND toward PC3 cells can be attributed to the presence of the pendant guanidine group as found in Arg-PEAs. The difference in PC3 cell cytotoxicity between Dex-PA-1X and Dex-PA-3X could result from an increase in the propylamine concentration. In Dex-PA-3X, all the 3 -OH groups in the dextran repeating unit were substituted by propylamine, while only one out of three -OH groups in the Dex-PA-1X repeating unit was substituted by propylamine. Similarly it was shown that a cationic dextran derivative with 25% amine substitution resulted in more cell death than 22% amine substitution at

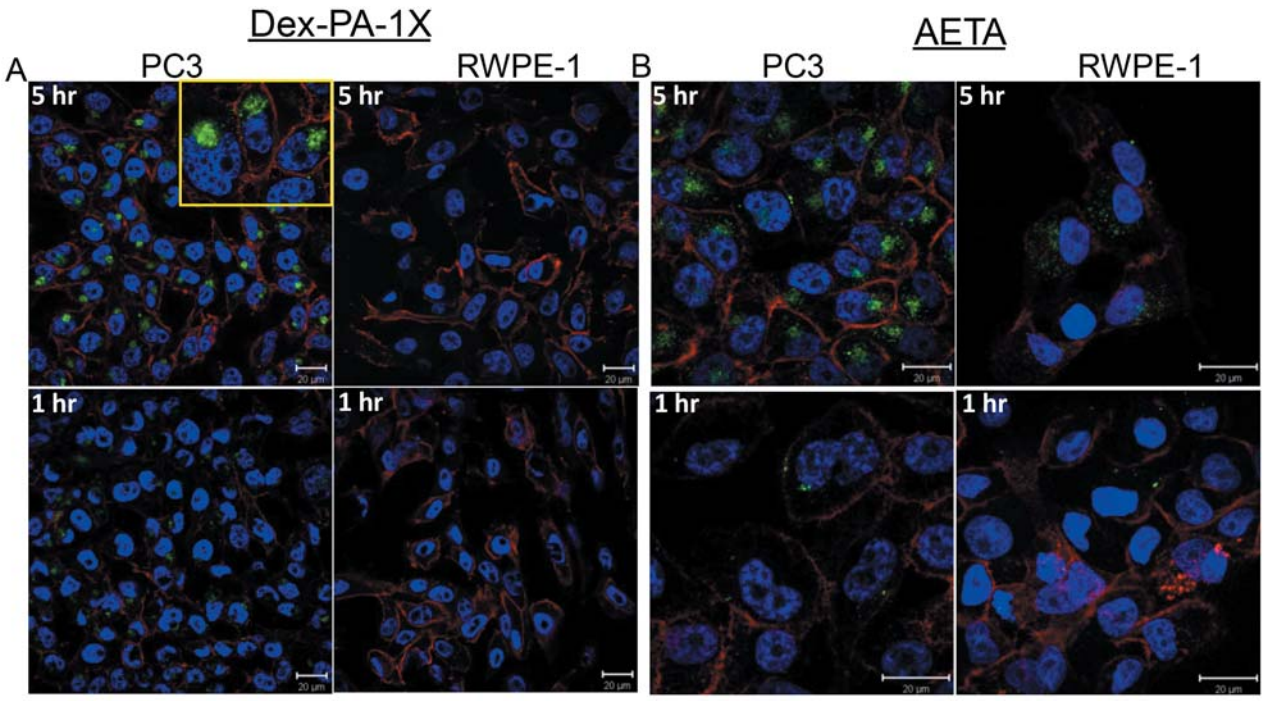

Figure 6. *Confocal microscopy showing the localization of Dex-PA-1X and PolyAETA in PC3 and RWPE-1 prostate cells. Both PolyAETA and Dex-PA-1X was used at 100 μg/ml. A) Localization of Dex-PA-1X at 1 and 5 h. Dex-PA-1X starts appearing in the cytoplasm of PC3 cells at 1 h and its concentration inside the cytoplasm increases with time. On the contrary, no Dex-PA-1X is seen in RWPE-1 cells at any time point used in the study. Red= EpCAM, Green= Dex-PA-1X. B) Localization of PolyAETA in PC3 and RWPE-1 cells at similar time points as in A. PolyAETA is concentrated more in PC3 cells at 5 h in comparison to RWPE-1 cells. Red= EpCAM, Green= PolyAETA.*

similar concentrations (11). Azab *et al*. also reported that the percentage binding of the cationic polymer was increased when the charge density of the cationic polymers was increased by substituting more cations and that this binding was reduced in the presence of neuraminidase (removes sialic acid) (36).

The cationic property of PolyAETA, a vinyl-based non-biodegradable synthetic cationic polymer, comes from the pendant quaternary ammonium salt which retains its cationic charge over most of the pH range. Raffaghello *et al*. reported that polyvinyl alcohol-based polymers also showed a potent cytotoxicity toward neuroblastoma and melanoma cell lines *via* the induction of caspase signaling followed by apoptosis (39). In contrast, AA-PEA polymers did not demonstrate cytotoxicity, which may result from the cationic nature of the pendant guanidine group of arginine, having a pKa of 12.5. This lack of adverse effect from the guanidine group is consistent with our screening data in which the -OH groups of the dextran, was substituted by the pendant guanidine group Dex-GND, which showed higher PC3 cell proliferation than the control. Meurling *et al*. also reported that free (unconjugated) guanidine compound showed no adverse effect on the tumor cell growth of bladder cancer cell lines even at mM concentrations (38).

The mechanism of cytotoxicity caused by cationic polymers is not fully-understood. Because of their growth inhibitory effects on PC-3 cells, we further evaluated cells treated with Dex-PA and PolyAETA. At lower concentrations of polymer, cell death was induced *via* activation of caspase signaling, whereas at higher concentrations, a significant amount of necrosis was observed. Several studies have shown that dextran-based polymers or drugs coupled to these polymers can be used to induce apoptosis or to inhibit proliferation of malignant cells (40-45). Marquez *et al*. showed a 100% cell death in PC3 cells using a cationic dextran as detected by fluorimetric cytotoxicity assay (11).

The increased cytotoxic and apoptotic effect of the polymers on PC3 cells in comparison with RWPE-1 cells may be due to the differential expression of sialic acid on their respective cell surfaces. Marquez *et al*. (2004) indirectly showed the presence of sialic acid on PC3 cells as determined by binding of the FITC-labeled lectin. However, no control cell line, such as normal prostate cells were used in that study (11). In the present study, confocal laser scanning microscopy data revealed the presence of both PolyAETA and Dex-PA-3X in the cytoplasm of PC3 cells to a larger extent than RWPE-1 cells. This also supports our data showing higher

cytotoxicity and apoptosis in PC3 cells in comparison to RWPE-1 cells. Our localization study, however, cannot validate Marquez *et al*.' findings that the cytotoxicity of these cationic polymers was due to their preferential binding onto cancer cell membranes that could disrupt cell proliferation, since we did not observe apparent cell membrane localization of the cationic polymers (11). Another study conducted by Azab *et al*. showed that SW620, a highly metastatic colon cancer cell line, demonstrated more binding of the cationic polymer than SW480, a less aggressive colon cancer cell line (36). Furthermore, in the presence of neuraminidase, the percentage of bound cationic polymer was markedly reduced in SW620 cells compared with SW480 cells.

Overall, the data suggest that cationic and water-soluble polymers may have the potential to differentially target malignant prostate cells *versus* non-malignant cells based on the differences in their cell surface charges. Further exploration of the use of these polymers as a drug-alone or as a vector for therapeutic drug delivery is required.

Acknowledgements

This study was partially supported by a seed grant from Cornell University Prostate Cancer Research Group; the Robert H. McCooey Sr. Cancer Research Fund; NIH Physical Sciences Oncology Center at Cornell (U54CA143876) and Department of Defense - Prostate Cancer Research Program (W81XWH-12-1-0124). We thank Drs. Jun Wu, G.M. Sun and Mr. M. Y. He for providing polymers.

References

1 Holmberg AR, Wilchek M, Marquez M, Westlin JE, Du J and Nilsson S: Ion exchange tumor targeting: A new approach. Clin Cancer Res *5*: 3056s-3058s, 1999.

2 Kawai T, Kato A, Higashi H, Kato S and Naiki M: Quantitative determination of N-glycolylneuraminic acid expression in human cancerous tissues and avian lymphoma cell lines as a tumor-associated sialic acid by gas chromatography-mass spectrometry. Cancer Res *51*: 1242-1246, 1991.

3 Kokoglu E, Sonmez H, Uslu E and Uslu I: Sialic acid levels in various types of cancer. Cancer Biochem Biophys *13*: 57-64, 1992.

4 Lin S, Kemmner W, Grigull S and Schlag PM: Cell surface alpha 2,6 sialylation affects adhesion of breast carcinoma cells. Exp Cell Res *276*: 101-110, 2002.

5 Lopez-Morales D, Reyes-Leyva J, Santos-Lopez G, Zenteno E and Vallejo-Ruiz V: Increased expression of sialic acid in cervical biopsies with squamous intraepithelial lesions. Diagn Pathol *5*: 74, 2010.

6 Seidenfaden R, Krauter A, Schertzinger F, Gerardy-Schahn R and Hildebrandt H: Polysialic acid directs tumor cell growth by controlling heterophilic neural cell adhesion molecule interactions. Mol Cell Biol *23*: 5908-5918, 2003.

7 Bondioli L, Ruozi B, Belletti D, Forni F, Vandelli MA and Tosi G: Sialic acid as a potential approach for the protection and targeting of nanocarriers. Expert Opin Drug Deliv *8*: 921-937, 2011.

8 Collard JG, Schijven JF, Bikker A, La Riviere G, Bolscher JG and Roos E: Cell surface sialic acid and the invasive and metastatic potential of T-cell hybridomas. Cancer Res *46*: 3521-3527, 1986.

9 Daniel L, Trouillas J, Renaud W, Chevallier P, Gouvernet J, Rougon G and Figarella-Branger D: Polysialylated-neural cell adhesion molecule expression in rat pituitary transplantable tumors (spontaneous mammotropic transplantable tumor in wistar-furth rats) is related to growth rate and malignancy. Cancer Res *60*: 80-85, 2000.

10 Daniel L, Durbec P, Gautherot E, Rouvier E, Rougon G and Figarella-Branger D: A nude mice model of human rhabdomyosarcoma lung metastases for evaluating the role of polysialic acids in the metastatic process. Oncogene *20*: 997-1004, 2001.

11 Marquez M, Nilsson S, Lennartsson L, Liu Z, Tammela T, Raitanen M and Holmberg AR: Charge-dependent targeting: Results in six tumor cell lines. Anticancer Res *24*: 1347-1351, 2004.

12 Sun G and Chu CC: Self-assembly of chemically engineered hydrophilic dextran into microscopic tubules. ACS Nano *3*: 1176-1182, 2009.

13 Sun G and Chu CC: Impregnation of tubular self-assemblies into dextran hydrogels. Langmuir *26*: 2831-2838, 2010.

14 Sun G and Chu CC: Biodegradable nanospheres self-assembled from complementary hydrophilic dextran macromers. Carbohydrate Polymers *86*: 910-916, 2011.

15 Zhong C and Chu CC: Acid polysaccharide-induced amorphous calcium carbonate (ACC) films: Colloidal nanoparticle self-organization process. Langmuir *25*: 3045-3049, 2009.

16 Zhong C, Wu J, Reinhart-King CA and Chu CC: Synthesis, characterization and cytotoxicity of photo-crosslinked maleic chitosan-polyethylene glycol diacrylate hybrid hydrogels. Acta Biomater *6*: 3908-3918, 2010.

17 Song H: L-arginine-based biodegradable poly (ester amide)s, their synthesis, characterization, fabrication, and application as drug and gene carriers. Ph.D. Thesis, August 2007.

18 Song H and Chu CC: Synthesis and characterization of A new family of cationic poly (ester amide)s and their biological properties. Journal of Applied Polymer Science *124*: 3840-3853, 2012.

19 Deng M, Wu J, Reinhart-King CA and Chu CC: Synthesis and characterization of biodegradable poly (ester amide) s with pendant amine functional groups and *in vitro* cellular response. Biomacromolecules *10*: 3037-3047, 2009.

20 Horwitz JA, Shum KM, Bodle JC, Deng M, Chu CC and Reinhart-King CA: Biological performance of biodegradable amino acid-based poly (ester amide) s: Endothelial cell adhesion and inflammation *in vitro*. J Biomed Mater Res A *95*: 371-380, 2010.

21 Wu J: Arginine and phenylalanine based poly(ester amide)s: Synthesis, characterization and applications. Ph.D. Thesis, Jan 2011.

22 Pang X, Wu J, Reinhart-King C and Chu CC: Synthesis and characterization of functionalized water soluble cationic poly(ester amide)s. Journal of Polymer Science Part A: Polymer Chemistry *48*: 3758-3766, 2010.

23 Yamanouchi D, Wu J, Lazar AN, Kent KC, Chu CC and Liu B: Biodegradable arginine-based poly(ester-amide)s as non-viral gene delivery reagents. Biomaterials *29*: 3269-3277, 2008.

24 Wu DQ, Chu CC and Chen FA: Cationic poly(VCL-AETA) hydrogels and ovalbumin (OVA) release *in vitro*. J Mater Sci Mater Med *19*: 3593-3601, 2008.

25 Chu CC: Novel biodegradable functional amino acid-based poly(ester amide) biomaterials: Design, synthesis, property and biomedical applications. Journal of Fiber Bioengineering and Informatics *5*: 1-31, 2012.
26 Wu J, Yamanouchi D, Liu B and Chu C: Biodegradable arginine-based poly(ether ester amide)s as non-viral DNA delivery vector and their structure – function study. Journal of Materials Chemistry *22*: 18983-18991, 2012.
27 Wu J, Mutschler MA and Chu CC: Synthesis and characterization of ionic charged water soluble arginine-based poly(ester amide). J Mater Sci Mater Med *22*: 469-479, 2011.
28 Wu J, Wu D, Mutschler MA and Chu C: Cationic hybrid hydrogels from amino-acid-based poly(ester amide): Fabrication, characterization, and biological properties. Advanced Functional Materials *22*: 3815-3823, 2012.
29 Thierry B, Merhi Y, Silver J and Tabrizian M: Biodegradable membrane-covered stent from chitosan-based polymers. J Biomed Mater Res A *75*: 556-566, 2005.
30 Lee SH, Szinai I, Carpenter K, Katsarava R, Jokhadze G, Chu CC, Huang Y, Verbeken E, Bramwell O, De Scheerder I and Hong MK: *In vivo* biocompatibility evaluation of stents coated with a new biodegradable elastomeric and functional polymer. Coron Artery Dis *13*: 237-241, 2002.
31 Joshi VB, Geary SM and Salem AK: Biodegradable particles as vaccine antigen delivery systems for stimulating cellular immune responses. Hum Vaccin Immunother *9*: 2584-2590, 2013.
32 Mogosanu GD and Grumezescu AM: Natural and synthetic polymers for wounds and burns dressing. Int J Pharm *463*: 127-136, 2014.
33 Sumitomo M, Milowsky MI, Shen R, Navarro D, Dai J, Asano T, Hayakawa M and Nanus DM: Neutral endopeptidase inhibits neuropeptide-mediated transactivation of the insulin-like growth factor receptor-akt cell survival pathway. Cancer Res *61*: 3294-3298, 2001.
34 Julien S, Lagadec C, Krzewinski-Recchi MA, Courtand G, Le Bourhis X and Delannoy P: Stable expression of sialyl-tn antigen in T47-D cells induces a decrease of cell adhesion and an increase of cell migration. Breast Cancer Res Treat *90*: 77-84, 2005.
35 Wang P: Altered glycosylation in cancer: Sialic acids and sialyltransferases. Journal of Cancer Molecules *1*: 73-81, 2005.
36 Azab AK, Kleinstern J, Srebnik M and Rubinstein A: The metastatic stage-dependent mucosal expression of sialic acid is a potential marker for targeting colon cancer with cationic polymers. Pharm Res *25*: 379-386, 2008.
37 Marquez M, Du J, Edgren M, Nilsson S, Lennartsson L, Hiltunen J, Westlin JE, Tammela T, Raitanen M, Laato M, Jonsson G and Holmberg AR: Development of dextran derivatives with cytotoxic effects in human urinary bladder cancer cell lines. Anticancer Res *22*: 741-744, 2002.
38 Meurling L, Marquez M, Nilsson S and Holmberg AR: Polymer-conjugated guanidine is a potentially useful anti-tumor agent. Int J Oncol *35*: 281-285, 2009.
39 Raffaghello L, Zuccari G, Carosio R, Orienti I and Montaldo PG: *In vitro* and *in vivo* antitumor activity of the novel derivatized polyvinyl alcohol-based polymer P10(4). Clin Cancer Res *12*: 3485-3493, 2006.
40 Zhang N, Khawli LA, Hu P and Epstein AL: Generation of rituximab polymer may cause hyper-cross-linking-induced apoptosis in non-hodgkin's lymphomas. Clin Cancer Res *11*: 5971-5980, 2005.
41 Malherbe S, Crepin M, Legrand C and Wei MX: Cytostatic and pro-apoptotic effects of a novel phenylacetate-dextran derivative (NaPaC) on breast cancer cells in interactions with endothelial cells. Anticancer Drugs *15*: 975-981, 2004.
42 Jiang M, Ouyang H, Ruan P, Zhao H, Pi Z, Huang S, Yi P and Crepin M: Chitosan derivatives inhibit cell proliferation and induce apoptosis in breast cancer cells. Anticancer Res *31*: 1321-1328, 2011.
43 Ma H, Li X, Yang Z, Okuno S, Kawaguchi T, Yagi S, Bouvet M and Hoffman RM: High antimetastatic efficacy of MEN4901/T-0128, a novel camptothecin carboxymethyldextran conjugate. J Surg Res *171*: 684-690, 2011.
44 Bigioni M, Parlani M, Bressan A, Bellarosa D, Rivoltini L, Animati F, Crea A, Bugianesi R, Maggi CA, Manzini S and Binaschi M: Antitumor activity of delimotecan against human metastatic melanoma: Pharmacokinetics and molecular determinants. Int J Cancer *125*: 2456-2464, 2009.
45 Araki Y, Mukaisyo K, Sugihara H, Fujiyama Y and Hattori T: Increased apoptosis and decreased proliferation of colonic epithelium in dextran sulfate sodium-induced colitis in mice. Oncol Rep *24*: 869-874, 2010.

Received May 27, 2014
Revised June 17, 2014
Accepted June 18, 2014