colloids and interfaces

MDPI

SUPPORTING INFORMATION

Multi-target inhibition of cancer cell growth by siRNA cocktails and 5-fluorouracil using effective piperidine-terminated phosphorus dendrimers

Aliaksei Ihnatsyeu-Kachan [1], Volha Dzmitruk [1], Evgeny Apartsin [2], Olga Krasheninina [2], Maksim Ionov [3], Svetlana Loznikova [1], Alya Venyaminova [2], Katarzyna Milowska [3], Dzmitry Shcharbin [1*], Serge Mignani [4,5], Maria Angeles Muñoz-Fernández [6,7], Jean-Pierre Majoral [8,9], Maria Bryszewska [3]

[1] Institute of Biophysics and Cell Engineering of NASB, Minsk, Belarus

[2] Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russia

[3] Department of General Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Poland

[4] Laboratoire de Chimie et de Biochimie Pharmacologiques et Toxicologique, Université Paris Descartes, PRES Sorbonne Paris Cité, CNRS UMR 860, Paris, France

[5] Centro de Química da Madeira, MMRG, Universidade da Madeira, Funchal, Portugal

[6] Networking Research Center on Bioengineering, Biomaterials and Nanomedicine, CIBER-BBN, Spain

[7] Laboratorio de Inmunobiología Molecular, Hospital General Universitario Gregorio Marañón, Madrid, Spain

[8] Laboratoire de Chimie de Coordination du CNRS, Toulouse, France

[9] Université de Toulouse, UPS, INPT, Toulouse, France

1. Circular Dichroism

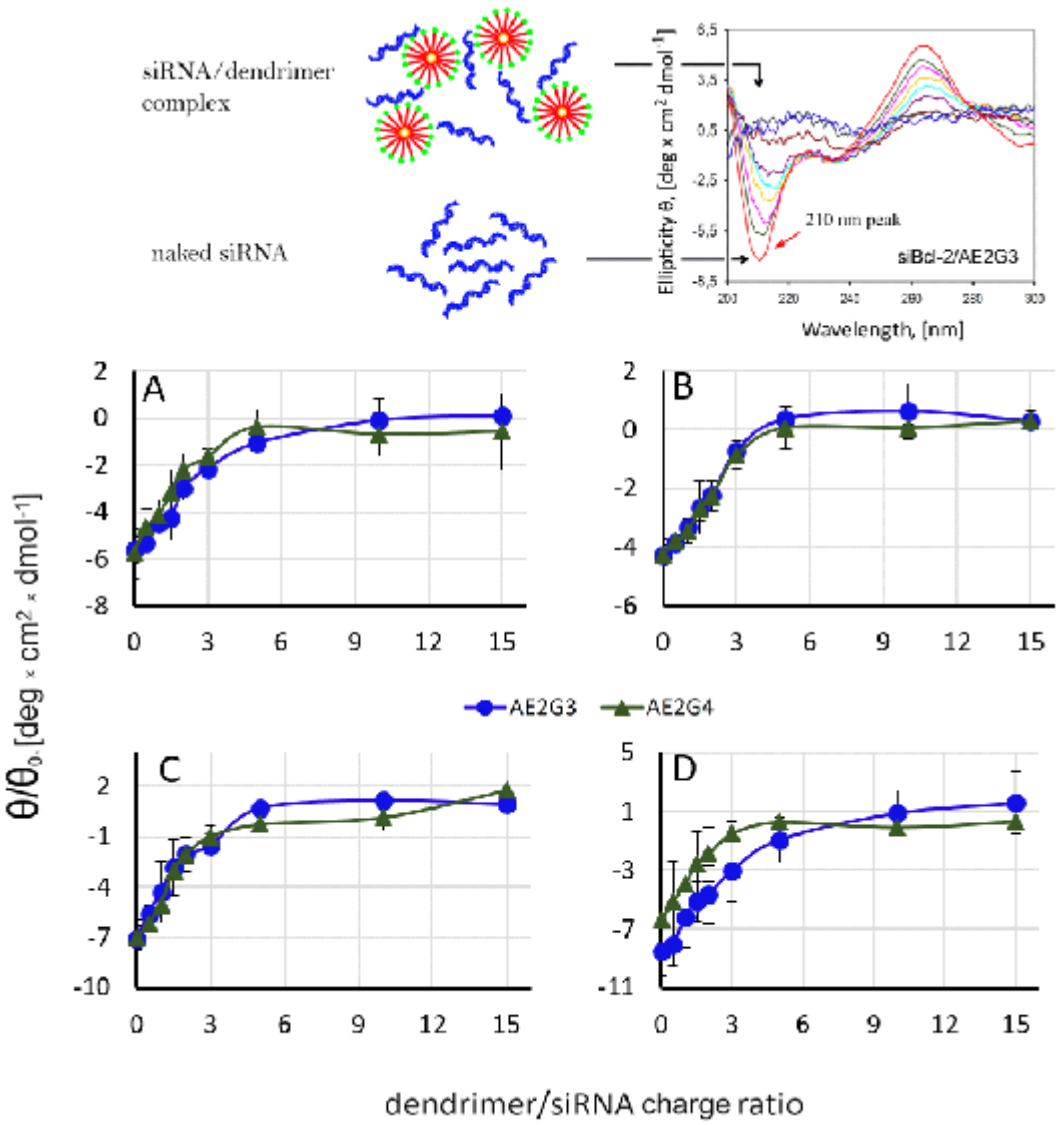


Figure 1. Changes in mean residue ellipticity at λ= 210 nm of the (A) - siBcl-xl, (B) - siMcl-1, (C) - siBcl-2, (D) - siScrambled siRNAs upon addition of AE2G3 (●) and AE2G4 (▲) dendrimers at rising dendrimer/siRNA charge ratios in 10 mmol/L Na-phosphate buffer, pH 7.4. [siRNA] = 1 uM. The results represent means ± SD from at least 3 independent experiments.

Top panel demonstrates the CD spectra of the siBcl-2 siRNA in the presence of increasing concentrations of AE2G3 dendrimer, bandwidth 1.0 nm, response time 2 s, scan speed 50 nm/min, step resolution 1 nm, n=3.

siRNAs' ellipticity at λ = 210 nm peak decreased with increasing dendrimer/siRNA charge ratio. For both dendrimers ellipticity reached a value close to 0 (Figure 1), and the changes were similar regardless of the generation. Reaching the plateau in changes of the molar ellipticity upon addition of dendrimers indicates the formation of stable saturated complexes between siRNAs and the dendrimers.

2. Measurement of zeta potential

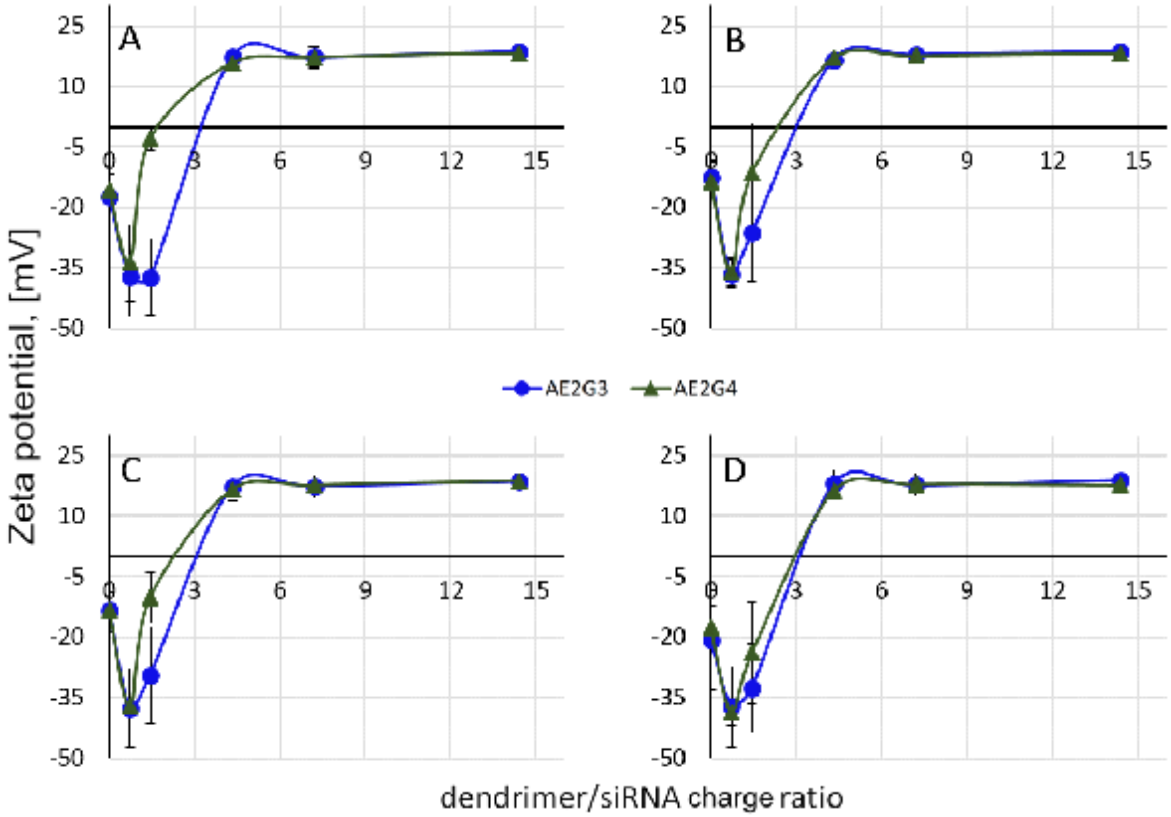
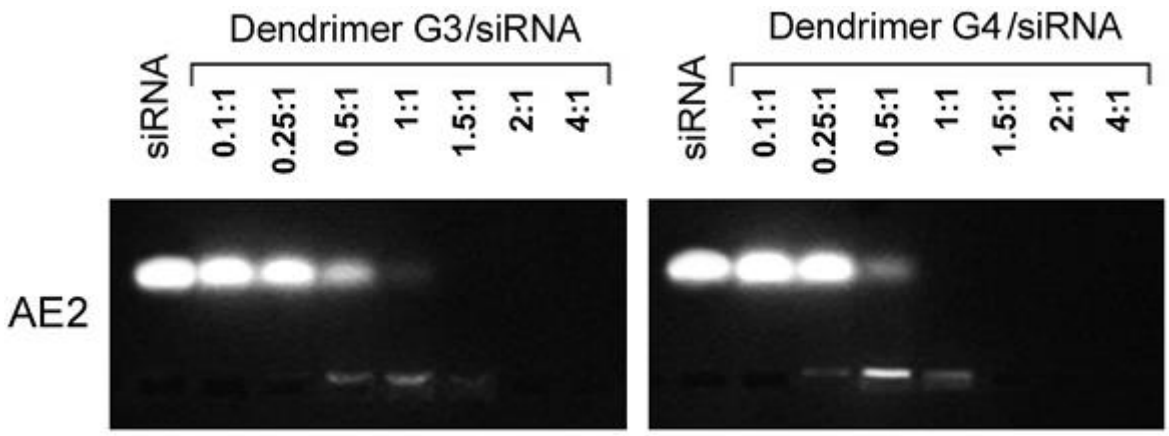


Figure 2. Zeta potential of the (A) - siBcl-xl, (B) - siMcl-1, (C) - siBcl-2, (D) - siScrambled siRNAs upon addition of AE2G3 (●) and AE2G4 (▲) dendrimers at rising dendrimer/siRNA charge ratios in 10 mM Na-phosphate buffer, pH 7.4. Results represent mean ±SD obtained from minimum three independent experiments.

3. Gel electrophoresis

Figure 3 shows the electrophoregrams with different patterns of migration of siBcl-xl (labelled with fluorescein) depending on the kind of dendrimer. Complexes were formed at different dendrimers/siRNA molar ratios with siRNA being mixed with increasing concentrations of Dendrimers AE2 (Fig. 3). Under the same conditions, dendrimers could fully retard siRNA mobility in gels, being complete when in excess.



siBcl-xl (labelled with fluorescein)/AE2G3; AE2G4. Molar ratios

Figure 3. Analysis of the formation of dendrimer/siRNA complexes. Dendrimers were complexed with fluorescein-labeled siRNA in 10 mmol/L Na phosphate buffer, pH 7.4. Complexes were analyzed by electrophoresis on 3% agarose gels with siRNA migration identified by labeled siRNA staining. The complexes were formed at different dendrimer/siRNA molar ratios. The first lane shows the migration of non-complexed siRNA.

To test a possible protective effect of dendrimers on siRNA against degradation by RNAse, agarose gel electrophoresis was used. Naked siRNA was completely digested in the presence of RNase A (Figure 4, second line). When siRNA was incubated with dendrimers, treatment with RNase did not lead to degradation, since addition of heparin led to its release from the complex.

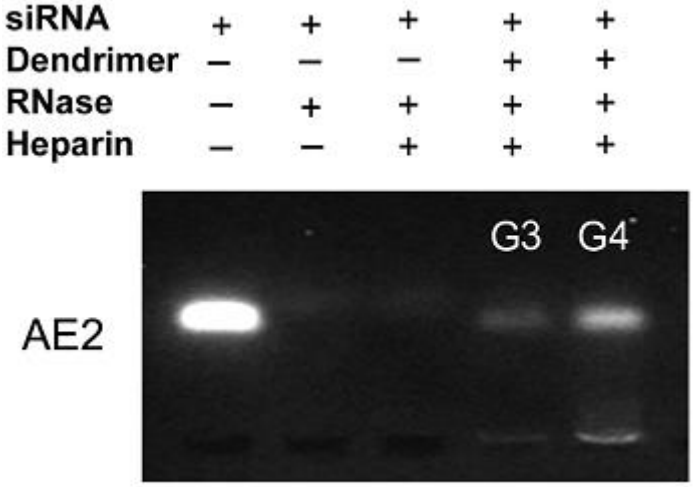


Figure 4. Complexes stability. Dendrimers have a protective effect on siRNA in the presence of RNase. The first lane shows migration of non-complexed siRNA. The second lane shows migration of siRNA previously incubated with RNase A. The third lane shows migration of siRNA previously incubated with RNase A in the presence of heparin (0.82 mg/ml). Next lines are the migration of siRNA due to its release from complexes in the presence

of heparin 0.082 mg/ml, representing the protective effect of dendrimers to siRNA against RNase.

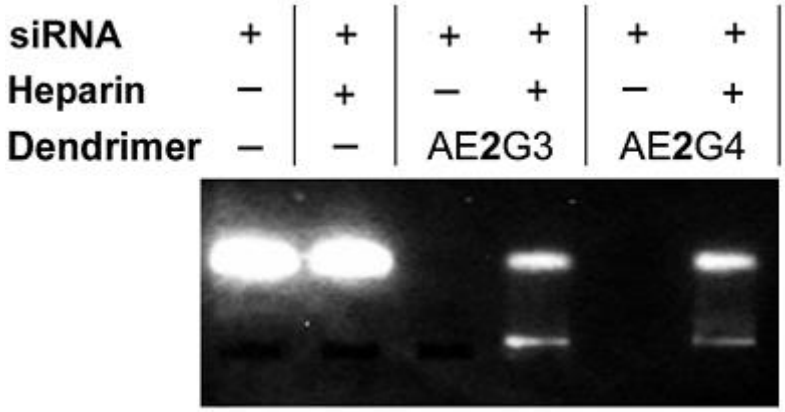


Figure 5. Release of the siRNA from dendriplex by addition of heparin (0.082 mg/ml). The first lane shows migration of non-complexed siRNA. The second lane shows migration of siRNA in the presence of heparin (0.082 mg/ml). Next lines are the migration of siRNA due to its release from complexes after addition of heparin 0.082 mg/ml, representing the release of siRNA from the complex due its interaction with heparin.

4. Atomic force microscopy

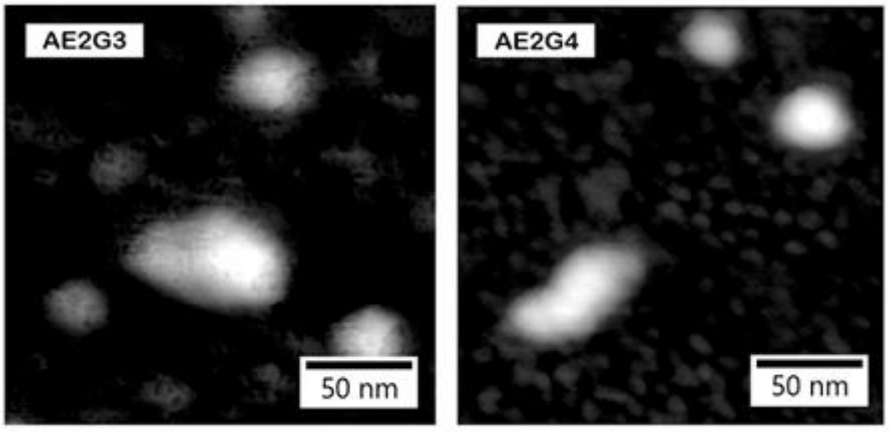


Figure 6. AFM images of siMcl-1/dendrimer mixtures composed using AE dendrimers. To obtain the fully saturated complexes, the charge ratios of dendrimers to siRNA were 10:1.

5. Confocal microscopy

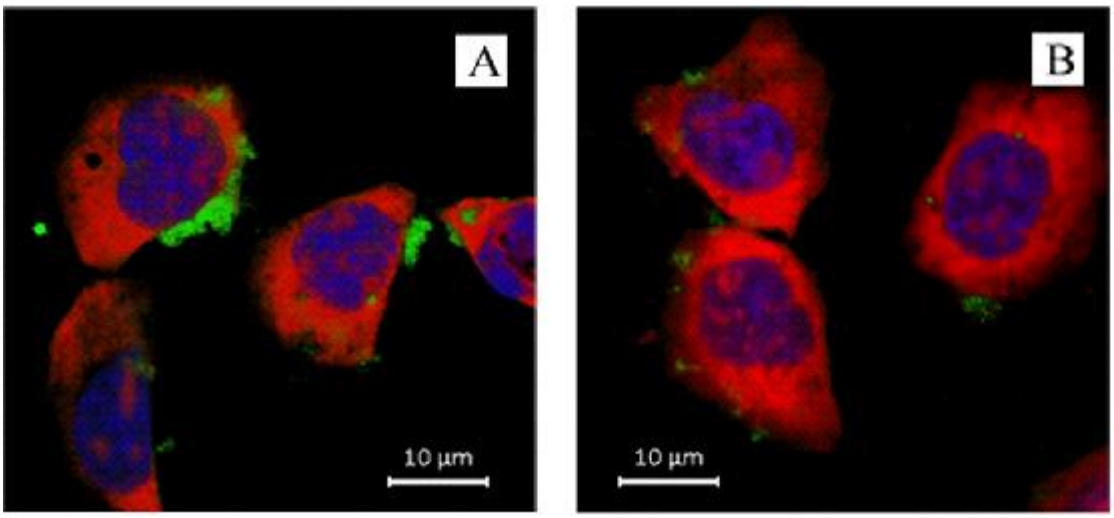


Figure 7. Representative confocal microscopy image of HeLa cells (cross-section) after 3 h incubation with 100 nM of fluorescein-labeled siBCL-xL in complex with: A– AE2G3 dendrimer, B – AE2G4 dendrimer. Dendrimer/siRNA charge ratio 10:1.