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Polyplex nanoparticles for cancer gene therapy

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Abstract. We synthesized and evaluated properties of the polyethylenimine (PEI)-polyethylene glycol (PEG)-TAT peptide polyplex nanoparticles. Variations in component ratios enabled to find their optimal combinations giving very high transfection efficacy (TE), up to ca. 100% for several cell lines. Investigations of subcellular transport kinetics and unpacking of the polyplex nanoparticles demonstrated positive correlation of TE with the cellular uptake rate of the nanoparticles and negative correlation with the rate constant of their unpacking within endo/lysosomal compartments in the living cells. Intratumoral administration of polyplexes carrying HSV*tk* and GM-CSF genes (with subsequent injection of ganciclovir) to S37 sarcoma-bearing mice resulted in significant inhibition of tumor growth (by 88%), metastasizing (by 79%), and prolongation of average life-span (by 83%). We have synthesized and investigated properties of new targeted PEG-PEI-based polyplexes containing MC1SP-peptide, a ligand specific for melanocortin receptors-1 overexpressed on melanoma cells. The targeted polyplexes caused significantly higher *in vitro* and *in vivo* transfection of melanoma tumor cells. The i.v. injected targeted polyplexes carrying sodium-iodide symporter (NIS) gene efficiently transfected *in vivo* melanoma tumors in melanoma-bearing mice which can be revealed by subsequent intravenous injection of $^{123}I^-$ and single-photon tomography thus demonstrating possible expediency of this approach for both diagnostic and therapeutic purposes.

Non-viral DNA delivery systems attract growing attention of not only researchers but also clinicians and now these systems are used in more than 33% of clinical trials [1]. Polyplexes, complexes of DNA and polycations of different nature, is a perspective non-viral delivery system which showed promising results both *in vitro* and *in vivo*. One of the most often used polymers for formation of polyplex nanoparticles for gene therapy purposes is a positively charged polyethylenimine (PEI). It is often modified by (polyethylene)glycol (PEG) which results in formation of a hydrophilic corona around the PEI/DNA core and reduction of ζ-potential of PEI-based polyplexes. We used PEI-PEG-containing polyplexes additionally modified with either a cell-penetrating peptide, TAT-derived peptide[1], in order to enhance their efficacy [2], or melanocortin receptor-1 specific ligand, MC1SP-peptide[2], in order to impart melanoma-cell specificity to them [3]. Their physico-chemical properties have been characterized earlier [2,3].

We tried to correlate some properties of the PEI-PEG-TAT-containing polyplex nanoparticles as well as the behavior of the nanoparticles in the cells with the transfection efficacy (TE) estimated using different cell lines [2]. Variations in PEG/PEI and N/P (PEI nitrogen/DNA phosphorus) ratios permitted us to find their optimal combinations giving very high TE, up to ca. 100% for several cell lines. We showed a statistically significant positive correlation between TE and a percentage of 50–75 nm fraction in polyplex nanoparticles estimated with atomic force microscopy. Surfaces of the TE dependence of both PEG/PEI and N/P turned out to be very similar in appearance for all investigated cell lines whereas maximum TEs were different. This difference was not caused by peculiarities of expression of genetic part of the polyplexes in different cell lines. Investigation of subcellular transport kinetics and unpacking of the polyplex nanoparticles demonstrated clear and statistically significant positive correlation of TE with the cellular uptake rate of the nanoparticles and negative correlation with the rate constant of their unpacking within endo/lysosomal compartments in the living cells. These results reveal specific directions for polyplex amendment.

The PEI-PEG-TAT-containing polyplexes were used to deliver HSV*tk*[3] and GM-CSF[4] genes into 4 tumor types in mice. The data obtained with one of these cancers, mouse S37 sarcoma on C_{57}Bl/6j×CBA F1 mice, are depicted on Fig. 1 and demonstrate encouraging results.

The PEI-PEG-MC1SP-containing polyplexes, targeted polyplexes, demonstrated receptor-mediated transfection of Cloudman S91 (clone M-3) murine melanoma cells which was more efficient than with non-targeted ones, i.e. lacking the MC1SP-peptide [3]. Packed targeted polyplexes appeared and accumulated in the melanoma cells 6 hours earlier than non-targeted ones. The targeted polyplexes enter into the nuclei of the melanoma cells more rapidly than non-targeted control and this difference may also be attributed to processes of receptor-mediated endocytosis. The targeted polyplexes caused significantly higher *in vivo* transfection of melanoma tumor cells after intratumoral administration than the non-targeted control. The targeted polyplexes carrying HSV*tk* gene and injected intratumorally more efficiently inhibited melanoma tumor growth and prolonged lifespan of DBA/2 tumor-bearing mice than non-targeted ones.

[1] Its amino acid sequence: GRKKKRRQRC.

[2] Its amino acid sequence: CGYGPKKKRKVSGSGSSIISHFRWG KPV, where melanocortin receptor-1 specific part is given in bold letters.

[3] HSV*tk*, Herpes simplex thymidine kinase gene, a gene often used for a so-called "suicide gene therapy" which includes delivery of the therapeutic transgene to the cancer cells and subsequent administration of a non-toxic substrate, ganciclovir, which is transformed into a highly toxic product by this thymidine kinase.

[4] GM-CSF, a gene encoding a cytokine, granulocyte-macrophage colony-stimulating-factor.

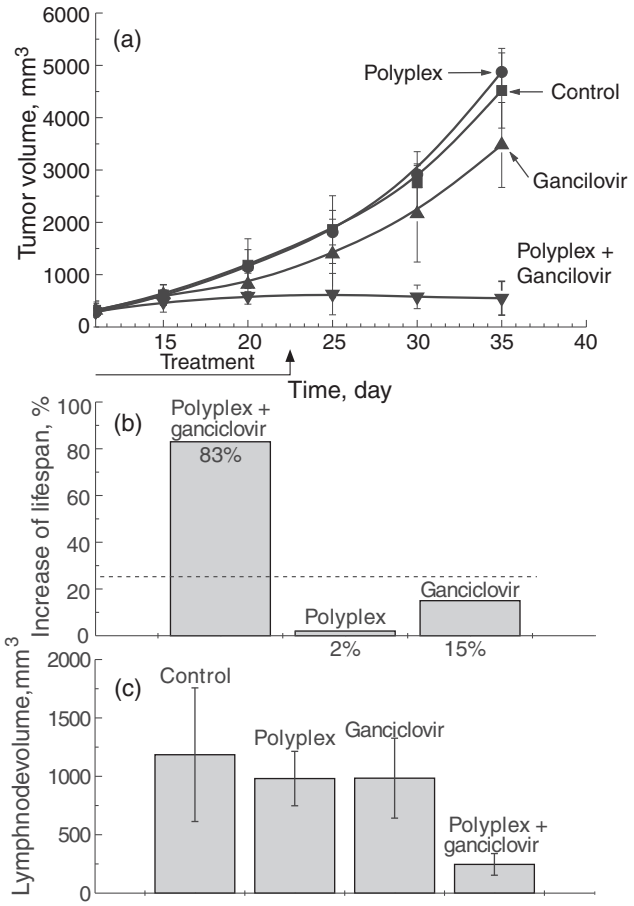

Fig. 1. Antitumor effect of suicide gene therapy of mice with subcutaneous sarcoma S37 tumors. Trice-repeated intratumoral injections of polyplexes with 4, 8 and 12 mg DNA of HSV*tk* and GM-CSF genes under the CMV promoter were performed on 7, 12, and 17 day after inoculation of 2×10^6 tumor cells. Ganciclovir treatment, 75 mg/kg twice a day for 15 days, was started 12 h after the polyplex injection. (a) tumor growth, (b) mouse survival, (c) lymph node growth reflecting tumor metastasizing.

We investigated microbiodistribution of intravenously injected targeted polyplexes with quantum-dot labeled DNA within melanoma tumors and normal tissues of mice using skinfold chambers permitting long intravital observations by multiphoton laser scanning microscopy. The obtained data (Fig. 2) uniquely demonstrated that the polyplex nanoparticles exit from capillaries and enter into tissue depth in tumors whereas in normal tissues there were registered only small amounts of them and only in the nearest vicinity of capillaries. These results together with the above data about higher efficacy and specificity of targeted polyplexes encouraged us to use these polyplexes for systemic administration.

After intravenous administration, the targeted polyplexes carrying NIS[5] gene efficiently transfected *in vivo* melanoma tumors in melanoma-bearing mice which can be revealed by subsequent intravenous injection of $^{123}I^-$ and single-photon tomography (Fig. 3). This result demonstrates possible expediency of this approach for both diagnostic and therapeutic purposes.

[5] NIS, a gene encoding sodium-iodide symporter, a protein transporting 2 sodium and 1 iodide ions into the cells where it is expressed. Normally, NIS is expressed in thyroid, stomach mucosa and salivary glands.

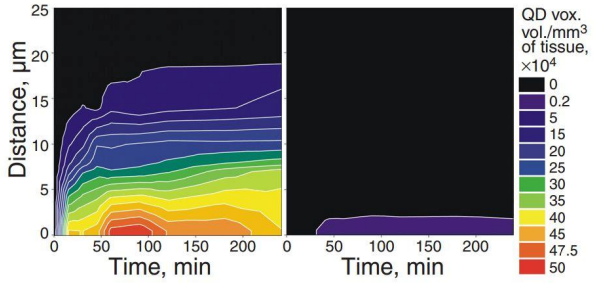

Fig. 2. Polyplex time-distance microbiodistribution in normal and tumor tissues of mice bearing Cloudman S91 (clone M3) murine melanoma tumors. Polyplexes with quantum-dot labeled DNA were injected intravenously. The legend shows quantum dot voxel volume per 1 cubic mm of tissue depending on time, minutes, after polyplex injection, and distance from the border of the nearest capillary, μm.

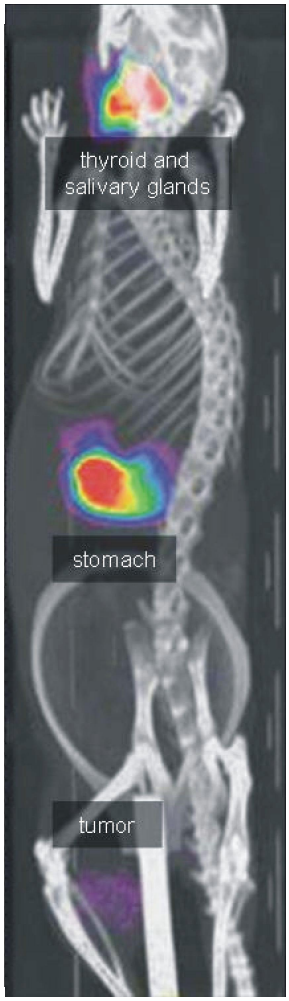

Fig. 3. SPECT/CT tomography of a DBA/2 mouse bearing Cloudman S91 (clone M3) melanoma. The mouse was transfected *in vivo* with polyplexes carrying NIS gene and 24 hours later was injected i.v. with 19 MBq of $^{123}I^-$.

Acknowledgements

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