Nanobiotechnology and Cancer Research

Bio-application of novel non-viral gene delivery nanocarrier

[1,2]Finiuk N., [2]Vitak T., [1]Filyak Y., [3]Mitina N., [3]Zaichenko O., [1]Stoika R.

[1] Institute of Cell Biology, Drahomanov St. 14/16, 79005, Lviv, Ukraine
[2] Ivan Franko National University of Lviv, Hrushevskyi St., 4, 79005, Lviv, Ukraine
[3] Lviv National Polytechnic University, 9 St. Yura Sq., 79013 Lviv, Ukraine
nataliyafiniuk@gmail.com

Gene delivery holds great potential in treatment of genetic and acquired human diseases. The success of non-viral gene therapy has been limited by inefficient gene delivery due to the presence of biological barriers for gene transfer, as well as high toxicity and immunogenicity.

Aim: Characteristics of highly efficient, safe, non-toxic, non-immunogenic gene delivery based on using novel olygoelectrolyte nanosized polymers.

Methods: Novel comb-like oligoelectrolyte nanocarriers were synthesized at Lviv National Polytechnic University via controlled radical copolymerization using oligoperoxide Cu^{+2} coordinating complexes. Electrophoresis, turbidimetry, MTT assay, Ames mutagenicity test were used. Transfection efficiency was determined by monitoring Green Fluorescent Protein expression.

Results: DNA-binding capacity of the polymers was evaluated and the best ones were tested for gene delivery. Transfection conditions were optimized. It was found that BG-2 oligoelectrolyte possessed 5-7 times higher gene delivery efficiency than other classical transfection methods. BG-2 was efficient in crossing cellular barriers due to high lipophilic activity and forming stable and small sized complexes with DNA. We observed that DNA was completely protected by BG-2 from the action of nucleases. The nanocarrier demonstrated low cytotoxicity and no mutagenic potencial towards treated cells.

Conclusions: Novel DMAEM-based olygoelectrolyte nanosized polymers are perspective for the use as nonviral gene delivery vectors. They are non-toxic and non-mutagenic. Their application is handy and time saving.

Keywords: Nonviral gene delivery, oligoelectrolyte polymeric nanocarrier.

This work was partially supported by CRDF and WUBMRC grants.

PDMAAm-coated γ-Fe2O3 nanoparticles for cell labeling

Zasońska B. A., [1]Boiko N.b, Horák D., [1]Stoika R.

Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic,
Heyrovského Sq. 2, 162 06 Prague, Czech Republic;
[1]Institute of Cell Biology NASU,
14/16, Dragomanov St. 79005 Lviv, Ukraine
zasonska@imc.cas.cz

Aim: Synthesis of poly(*N,N'*-dimethylacrylamide) (PDMAAm)-coated γ-Fe_2O_3 core-shell nanoparticles (NPs) by „grafting from" method and their use for mammalian cell labeling.
Methods: Superparamagnetic maghemite (γ-Fe_2O_3) nanoparticles were synthesized by co-precipitation of $FeCl_2$ and $FeCl_3$ with ammonium hydroxide that was followed by oxidation of magnetite with sodium hypochlorite. Surface of γ-Fe_2O_3 nanoparticles was functionalized by three different initiators [2,2'–azobis (2-methylpropionamidine) dihydrochloride, 4-cyano-4-{[2-cyano-3-(*N*-hydroxycarbamoyl)-2-methylpropyl]azo}pentanoic acid and 2,2'-azobis(*N*'-hydroxy-2-methylpropanimidamide)]. "Grafting-from" method was then applied for the attachment of PDMAAm on the NP surface. Both uncoated and PDMAAm-coated γ-Fe_2O_3 nanoparticles were thoroughly characterized by scanning and transmission electron microscopy, Fourier-transform infrared spectroscopy (FT-IR), dynamic light scattering, zetasizer, and elemental analysis. Murine macrophages of J774.2 line were used as a model to investigate the engulfment of the NPs by the cells using light and fluorescence microscopy. Cytotoxicity of the NPs was also determined.
Results: Maghemite NPs (~ 9 nm) with a rather narrow size distribution (characterized by polydispersity index = 1.24) were synthesized by wet co-precipitation method and oxidation. Surface of the γ-Fe_2O_3 nanoparticles was functionalized with different initiators whose presence was confirmed by FT-IR spectroscopy. Properties of the NPs did not change substantially after the functionalization. Further polymerization produced PDMAAm-coated γ-Fe_2O_3 nanoparticles. While their size in dry state increased to 13-16 nm, the hydrodynamic diameter was also higher (200 nm) compared with that of the uncoated NPs (113 nm). The NPs formed a stable colloid in aqueous solutions, as documented by the zeta–potential equal -51 mV. PDMAAm-coated γ-Fe_2O_3 nanoparticles were then investigated in the *in vitro* cell experiments in terms of their engulfment by murine macrophages at 30 min, 1 h, 2 h, 3 h, or 24 h of cell culturing. The NPs were relatively non-toxic for the cultured cells. Their engulfment was very efficient and already after 3 h treatment, a majority of PDMAAm-γ-Fe_2O_3 nanoparticles was engulfed by the macrophages. Effect of the mode of NP modification on cell engulfment is discussed.
Conclusions: Superparamagnetic NPs were successfully prepared by co-precipitation, and they were coated with PDMAAm using „grafting-from" the initiator-functionalized particle approach. The PDMAAm γ-Fe_2O_3 nanoparticles formed a stable aqueous colloid. Cell experiments proved that the NPs were not cytotoxic. The PDMAAm-γ-Fe_2O_3 nanoparticles were very quickly engulfed by murine macrophages of J774.2 line. Thus, they show high potential for use in diagnostics of phagocytic activity. Delivery of various significant biomedical substances, e.g., specific proteins and medicines, by using such NPs can be also anticipated.
Supported by GA CR P206/12/0381.

Structure-activity relationships of landomycins

[1]Panchuk R. R., [1,2]Lehka L. V., [3]Shaaban Kh. M., [3]Rohr J., [2]Ostash B. O., [2]Fedorenko V. O., [1,2]Stoika R. S.

[1]Institute of Cell Biology NASU,
79005, Lviv, Drahomanov Str 14/16
[2]Ivan Franko Lviv National University, Biology Faculty,
79005, Lviv, Hrushevskogo Str 4
[3]University of Kentucky, College of Pharmacy,
789 S. Limestone St. Lexington
rpanchuk@ukr.net

Aim: In-depth study of molecular mechanisms of anticancer activity of 7 members of landomycin (L) family (LA, 11-deoxyLA, LB, 11-deoxyLB, LE, LD, 11-deoxyLD), and identification of key motifs in chemical structure that are responsible for their antineoplastic potential.
Methods: Annexin V/propidium iodide staining – for quantitative measurement of apoptotic/necrotic cells, DAPI staining – for qualitative analysis of chromatin fragmentation, Western-blot analysis and flow cytometry – for cell cycle measurement, caspase inhibition assays and Western-blot analysis on a panel of 20 proteins involved in apoptosis – for identification of molecular targets of Ls in target cells.
Results: We found that elimination of C11-OH group in the aglycon structure significantly decreased anticancer potential of Ls, and the most drastic difference was observed with Ls with 6 sugar residues: while the IC_{50} of LA is 0.3 μM, the IC_{50} of 11-deoxy LA is only 3 μM. All Ls with 11-OH group at the aglycon specifically blocked tumor cell progression in G1 phase, in contrast to chemically related anticancer drug doxorubicin which caused G2/M block. L-induced G1-block was p21- and p27-dependent. Deoxy Ls did not possess such specificity. The activation of effector caspase-7 and cleavage of its substrate PARP-1 took place already in 3 h after starting LE treatment of Jurkat T-leukemia cells, while activation of the initiator caspases-2, -8, -9,-10 and mitochondrial proteins Bid, Bax, AIF, Cytochrome *C* took place much later – 9-12 h. We did not find any significant activation of caspase-3 in tumor cells by LE, while doxorubicin activated it.
Conclusions: Key structures in the molecule of landomycins responsible for their anticancer activity are three or more saccharide residues in glycosidic chain, mandatory terminal residue of L-rhodinose, and presence of C11-OH group in aglycon part. Effector caspase-7 is a potential target of landomycins. Such targeting might allow these drugs effectively killing tumor cells defective on other apoptotic proteins and, thus, resistant to chemotherapy.
Keywords: landomycins, tumor chemotherapy, apoptosis, caspases