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Case Study

Optimization of the kinetics of siRNA desorption from the surface of silicon nanoparticles

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Abstract

Oncological diseases are one of the most significant medical and social diseases in most countries of the world. Over the past decades, the search and development of new drugs, treatment regimens and methods of molecular diagnostics of malignant neoplasms remains relevant. In turn, an important goal of molecular genetic research is to suppress the expression of genes responsible for the development of tumors. The key targets taken into account in the development of antitumor drugs are proteins involved in carcinogenic changes in the cell. One of the promising molecular targets for the development of medicinal compounds in targeted therapy of tumor diseases is poly(ADP-ribose)polymerase 1 (PARP1). A potential way to inhibit PARP1 even at the stage of protein translation is RNA interference due to small interfering RNAs (siRNAs). For the penetration of siRNAs into the target cell, it is necessary to develop a method of their transportation controlled in space and time. An actual direction for solving this problem is the use of highly stable porous silicon-based nanoparticles. In the current study, in order to increase the functionality of nanoparticles, their surface was modified with various agents (functionalization), providing increased efficiency of drug loading and more uniform release.

More Information

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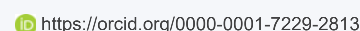
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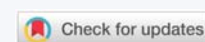

Introduction

One of the promising molecular targets for the development of medicinal compounds in targeted therapy of tumor diseases is poly(ADP-ribose)polymerase 1 (PARP1) [1,2]. The gene of the same name encoding this protein has increased expression in a number of oncological diseases, therefore it is considered an important molecular target in the development of antitumor agents.

Poly(ADP-ribosyl) action is a reaction that occurs when a cell responds to genotoxic stress. This process, being one of the posttranslational modifications of nuclear proteins, occurs in the presence of enzymes of the PARP poly(ADP-ribose) polymerase family, which play the role of recognizing breaks. The activity of members of the PARP family (PARP1 and PARP2) is associated with cellular signaling pathways. During poly(ADP-ribosyl)ation they ultimately contribute to changes in gene expression, the amount of RNA and proteins, as well as the localization and activity of proteins that mediate signaling responses. It is also crucial for a wide range of physiological and pathological responses and, thus, is a good target for chemical therapy of a number of diseases [3]. Preclinical data suggest that PARP inhibitors can enhance the effects of radiation therapy in certain types of tumors, namely: lung, colorectal cancer, head, and neck cancer, glioma, cervical cancer and prostate.

To disrupt the repair process in tumor cells, it is necessary to take into account the mechanisms of action of PARP inhibitors. PARP1 controls several DNA repair pathways: excision repair of bases (base excision repair, BER) and nucleotides (NER), mismatch repair (MMR), repair of double-stranded breaks using homologous recombination (HR) and non-homologous end joining (NHEJ) [4,5].

The most popular are biochemical methods of inhibiting PARP 1, such as enzymes and other agents. However, a potential way to reduce the influence of this factor even at the stage of protein translation is RNA interference.

RNA interference is the process of suppressing gene expression, which is carried out by small interfering RNAs (siRNAs). These are short RNAs that bind to the matrix RNA, thereby inhibiting the translation process [6].

For the penetration of siRNAs into the target cell, it is necessary to develop a method of their transportation controlled in space and time. A promising direction for solving this problem is the use of highly stable porous silicon-based nanoparticles (NPS) [7-16].

The issue of the genotoxicity of nano derivatives remains insufficiently investigated, and the methodology for assessing their genotoxic effects and predicting the genotoxic risk of human use has not yet been developed. The assessment of the genotoxicity of nanoparticles is fragmentary and contradictory and does not provide a prediction of the genotoxic risks of exposure of nanomaterials to humans.

Silicon nanoparticles belong to ceramic nanomaterials, along with aluminum and titanium nanoparticles. Most often they are made of porous material and are very common as carriers of medicines during antitumor therapy. The vast majority of silicon nanoparticles, including those protected by one or another coating, release more than 90% of therapeutic RNAs within 10-15 hours. The functionalization of polyethyleneimine (PEI) and (2-aminoethyl)-3-aminopropyltrimethoxysilane (DEMO-P) nanoparticles allows direct interaction with cancer cells and effective penetration into its microenvironment compared to traditional delivery systems [17].

The aim of our work was to optimize silicon nanoparticles for delivering small interfering RNAs against the PARP1 gene and ensuring their controlled desorption.

Materials and methods

To conduct the siRNA loading study, we used mesoporous NPS with a size of 120 nm. The study used siRNAs against one of the key transcription factors (PARP1). Nanoparticles were obtained by laser ablation and fragmentation in liquid. Laser ablation in liquid makes it possible to synthesize new ultrapure nanomaterials (without side contamination), which can make a decisive contribution to biomedical applications. In order to increase the functionality of the NPS, their surface was modified with various agents (functionalization), providing increased efficiency in loading the drug and more uniform release.

The experiment used untreated nanoparticles, as well as nanoparticles functionalized with polyethyleneimine (PEI) and (2-aminoethyl)-3-aminopropyltrimethoxysilane (DAMO-P) to study the possibility of loading RNA.

After that, the siRNA was loaded into the pores of the nanoparticles (in the case of untreated NPS) or onto their surface due to chemical bonds (in the case of functionalized NPS). For untreated nanoparticles, as well as for nanoparticles functionalized by PEI, there were 4 loading modes with ultrasound duration of 30, 60, 120 and 240 seconds.

The most important indicator for evaluating the effectiveness of nanoparticles as a means of transporting drugs is the desorption kinetics, that is, the rate of release of the drug (in the specific case of siRNA) from nanoparticles or from their surface.

To identify the kinetics of siRNA desorption from the surface of silicon nanoparticles, a regular concentration measurement was carried out. Measurements were performed on a NanoDrop ND-1000 spectrophotometer. Immediately before the experiments, suspensions of nanoparticles were subjected to ultrasonic treatment to eliminate the agglomerates formed during storage. The concentration of nanoparticles in suspensions was measured by the gravimetric method: by drying a certain volume of suspensions with an unknown concentration and measuring the mass of the dried substance.

Statistical analysis of data was performed using RStudio for Windows and Microsoft Excel. The nonparametric Chi-Square criterion is used to assess the reliability of the results [18].

Results

The study showed that silicon nanoparticles without additional modification (pSi) demonstrate an extremely low level of siRNA loading (about 1%), which is due to the retention of siRNA solely due to silicon pores. Loading siRNA onto silicon nanoparticles functionalized with PEI-pSi showed significantly higher efficiency (up to 10%). This is explained by the chemical binding of siRNA and the imino group in PEI. The insufficiently high loading rate is due to the small amount of PEI on the pSi surface.

The most optimal duration of ultrasonic treatment of a solution of nanoparticles and siRNA is 60 seconds. During this time, additional dispersion of nanoparticles occurs, and RNA almost does not degrade. This treatment provides the longest and most uniform yield of siRNA.

The functionalization of nanoparticles with 2-aminoethyl-3-aminopropyl-trimethoxysilane (DAMO-pSi) makes it possible to load more than 90% of siRNA during ultrasonic processing, which is almost 10 times more efficient than when processing PEI. Loading without ultrasound demonstrates significantly lower efficiency, which is explained by the aggregation of nanoparticles. When using DAMO-pSi, a sharp desorption intensity is observed (within 24 hours), which is less effective for RNA interference.

Discussion

Loading siRNA onto silicon nanoparticles functionalized with polyethyleneimine (PEIpSi) showed significantly higher efficiency (up to 10%). This is explained by the chemical binding of siRNAs and specifically the groups within PEI. The insufficiently high loading rate is due to the small amount of PEI on the pSi surface. The functionalization of nanoparticles with 2-aminoethyl-3-aminopropyl-triethoxysilane (DEMO-

pSi) allows loading more than 90% of siRNA during ultrasonic processing, which is almost 10 times more efficient than when processing PEI. Loading without ultrasound demonstrates significantly lower efficiency, which is explained by the aggregation of nanoparticles. When using DEMO-pSi, a sharp desorption intensity is observed (within 24 hours), which is less effective for RNA interference.

Conclusion

Small interfering RNAs have a great potential for wide application in biomedicine, in particular, in the treatment of malignant diseases [19]. PARP1, a key DNA repair protein, and the most important transcription factor is overexpressed in tumor cells, and therefore is a promising target for RNA interference. In the course of the study, the design of the siRNA sequence against PARP1, which does not affect other genes, was carried out.

For the transport of siRNA, nanoparticles of porous silicon are used as a biocompatible and biodegradable material. Various methods of surface coating and functionalization are used to absorb siRNA nanoparticles by target cells. The nature of the coating also determines the kinetics of the release of siRNA from nanoparticles [20].

Functionalization of silicon nanoparticles with polyethyleneimine demonstrates a low degree of loading but is a good indicator of desorption. When treated with silane, the loading efficiency is much higher, but there is a sharp release of siRNA. The optimal ultrasound treatment time is 60 seconds. The use of untreated nanoparticles is ineffective due to the low level of loading.

The results obtained indicate that the delivery of siRNA to target cells using treated silicon nanoparticles is a promising method for the treatment of oncological diseases. However, to confirm the effectiveness of such delivery, it is necessary to refine the methods of nanoparticle functionalization and RNA loading, as well as the use of molecular methods to determine the level of gene expression.

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