COMBINATION OF ANTICANCER SHORT RNA COCKTAILS WITH NANOMATERIALS AS A NOVEL TOOL TO TREAT CANCER CELLS

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Gene therapy is one of the most effective ways to treat tumor. One of the new directions of gene therapy is the suppression of malignancy arising in normal cells. During transformation, normal cells start to propagate uncontrollably, loosing the ability to undergo apoptosis, thus resulting in malignancies formation. The regulation of apoptosis in cells is implementedby the family of Bcl-2 proteins [1], which is divided into pro-apoptotic and anti-apoptotic proteins. The group of apoptosis inhibitors include Bcl-2, Bcl-xL, Bcl-w, A-1 and Mcl-xL. Anti-apoptotic proteins expression can be blocked at post-translational level by means of RNA interference (RNAi) – a process of selective gene silencing .RNAi effectors – siRNA (small interfering RNA)or microRNA are able by means of cellular machinery to arrest or cleave mRNA, responsible for the key cancer protein expression [2].

A major limit of such gene therapy application is effective delivery of nucleic acids siRNA into the target cells [3]. Nakednucleic acids undergo degradation by endogenous enzymes andare unable to penetrate cellular membranes owing to their largesize and high negative charge density. Specific and effective delivery of genetic material can be provided by a wide range ofviral and non-viral delivery systems. The viral systems are moreeffective but they are too costly and provide some critical side-effects such as high immunogenicity and carcinogenicity in vivo. Synthetic (non-viral) systems are comparatively less effective but more flexible andsafer[4]. Among large number of nanosynthetic materials dendrimers are highlighted for gene delivery due to their monodispersity, predetermined tree-like structure, stability, low viscosity and the large number of charged end groups. Dendrimers are synthetic polymers with a diameter of 3–10 nm. Cationic dendrimers can complex with nucleic acids by self-assembly, making complexes called “dendriplexes” [5].

In current research study different dendrimers were tested in terms to perform delivery system for different RNAi effectors. Their efficiency to contain survival rate of cancer cell lines were evaluated by applying mixtures of different RNAs as well as a singleshort RNA.

Here we represent experimental results on dendriplexes formed by 3 chemically different groups of dendrimers: PAMAM, carbosilane and phosphorous-containing, complexed with small RNAs designed against anti-apoptotic proteins Bcl-2, Bcl-xLand Mcl-1. The dendriplexes were characterized by such techniques as fluorescence analysis, zeta potential, dynamic light scattering (DLS), circular dichroism (CD), and transmission electron microscopic (TEM). Gel electrophoresis in the presence of RNase-A allowed us to answer a fundamental question as to whether dendrimers protect siRNA from degradation by nucleases.We also used a dendriplex disassociation assay involving a polyanionic agent–heparin – to check their ability to release siRNAs and monitor their structure after disassociation [6].

It was revealed all dendrimers form stable complexes with small acting RNAs by self-assembling in up to 1μm in size. In contrast to others phosphorous dendrimers are insensitive to heparin impact thus indicating not only electrostatic interaction within the dendriplex.

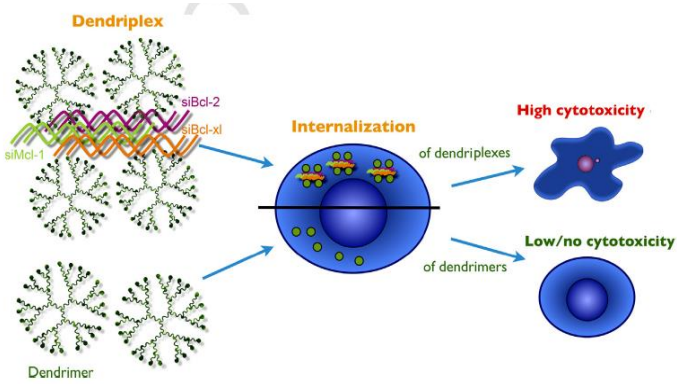

Figure 1 – A schematic representation of the mode of action of dendriplexes, including cocktail of different siRNAs (reprinted from [6])

The findings on complex formation show the potential of dendrimers as carriers of anticancer siRNA into cancer cells, thereby creating an alternative non-viral delivery system for BCL family siRNAs to target cells in gene therapy [6].

Cytotoxicity studies on HeLa (monolayer cervical cancer) and HL-60 (suspension promyelocytic T-leukemia) cell lines first showed up synergism of siRNAs action, targeted against different anti-apoptotic proteins, to be very effective when delivered by cationic dendrimers (figure 1), while anticancer action of each single siRNA was much less or even insignificant (as it was for HL-60). In our experiments the cytotoxicity of siRNA/dendrimer complexes has a dual naturebased on three mechanisms: apoptosis, autophagy and necrosis, siRNA induced apoptosis and dendrimers themselves may induce all above cell deaths depending on dendrimer's nature [7].

In summary, we report the usefulness of cationic dendrimers (PAMAM, CPD, CBD) as vectors for anticancer siRNAs. Phosphorous and PAMAM dendrimers were shown to facilitate siRNA intracellular penetration at a high rate, but reveled also high toxic effect, while carbosilane dendrimers were moderatein internalization efficacy with low toxic side effect. Both of the obtained systems can be used for gene therapy.

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