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Chimeric nucleolin aptamer with survivin DNAzyme for cancer cell targeted delivery

Nithya Subramanian, Jagat R Kanwar, Akilandeswari Balachandran, Rupinder K Kanwar, Vikas Khetan, Subramanian Krishnakumar

Chimeric aptamer-DNAzyme conjugate was generated for the first time using nucleolin aptamer (NCL-APT) and survivin Dz (Sur_Dz) and exerted targeted killing of cancer cells. The proof of concept of using aptamer for the delivery of DNAzyme can be applied on other cancers types to target survivin in cancer cell specific manner.

Deoxyribozyme, also known as DNAzyme (Dz), catalytic DNA or RNA enzyme, is single stranded synthetic DNA molecule which is catalytically active on nucleic acid targets. DNAzyme is more stable, easy to synthesis and less expensive than ribozyme, siRNA and antisense oligonucleotide, and hence is preferred over other targeting approaches. Several approaches have been used for Dz delivery including poly-L-Lysine (PLL) and Poly(lactic-co-glycolic acid) (PLGA) microspheres, transferrin modified polyplexes, dendrimers and nanoparticulate systems. Despite these systems, efficient target specific Dz delivery is not yet achieved.

Aptamers (APTs) are synthetic DNA or RNA oligos that possess unique secondary structures and binds target molecules with high specificity and affinity. APTs when chimerized, serve as cargoes for efficient and cell specific delivery of drug molecules, siRNA, proteins, radionuclides and nano structures. A DNA APT against nucleolin (NCL) AS1411 is FDA approved and was earlier used for delivery of splice switch oligos, siRNA and photoreactive drug, TMPyP4. NCL is a shuttle protein specifically expressed on cancer cell surface and acts as better target for cancer. NCL-APT in addition to its cancer cell specificity, also possess functional activities such as arrest of cell cycle and cytostasis.

Inhibitors of apoptosis family proteins (IAPs) are of interest in cancer targeting due to their role in preventing from apoptosis onset. Survivin (BIRC5 represented as Sur), an IAP is expressed in the cancer cells and is found to be highly overexpressed in the cytoplasm, mitochondria as well in nucleus, inhibits apoptosis of cancer cells and allows faster growth. Also survivin is reported to be associated with chemo and radiotherapy resistance, tumor recurrence and shorter patient survival. Survivin cross talks with many signaling pathways and mediates oncogenic role in various cancers and in retinoblastoma (RB). RB is a childhood eye cancer, represents about 4% of total pediatric malignancies. It is caused by retinoblastoma gene mutation or inactivation in both alleles of the retinoblast leading to changes in cell cycle and apoptosis regulators. Survivin protein is highly expressed in RB tumors and secreted in serum of RB patients. This makes this protein one of the best targets for therapy in RB.

In the present study, we utilized the Dz targeting survivin (Surv_Dz) that cleaves survivin mRNA efficiently in time and dose dependent manner in pancreatic carcinoma. As delivery of the oligonucleotides and toxicity of the carriers are to be analyzed, we hypothesized and tested our proof of concept of delivery of Surv_Dz using NCL-APT. Earlier, APT-siRNA chimeric construct, APT-ribozyme constructs were reported, also APT-Dz for the sensing of hemin, ochroatoxin A, adenosine and H$_2$O$_2$ form cancer cells. As there were reports lacking on the above concept, our study began with the NCL-APT, Surv_Dz chimerization, for targeted cancer cell delivery of Dz with uncompromised functional activity of the Dz.

In order to conjugate the NCL-APT with Surv_Dz, the NCL-APT was synthesized with poly T linker at the 5’ end followed by the complementary bases to Surv_Dz. This NCL-APT-linker was used for the conjugation with DZ using denaturing and annealing principle (details in supplementary). APT-Dz conjugate (NCL-APT-Sur_Dz), mutant APT-Dz conjugate (NCL-APT-Sur_mDz) and fluorescent APT-Dz conjugate (NCL-APT-Sur_fDz) conjugates were subjected to electrophoresis on agarose gel. The stability under in vitro conditions was tested by incubating the conjugates and Surv_Dz in 1X PBS containing 10% FBS and 1X PBS alone at 37°C upto 72 h. Cellular uptake in Y79, WERI-Rb-1 (neoplastic cell lines derived from RB tumors) and MIO-M1 (non-neoplastic müller glial cell derived cell line) cells were performed for checking the efficiency of the conjugate upon chimerization. Fluorescent Dz (fDz) was used for imaging the uptake of chimera and delivery of Dz into cells. The functional activity of conjugates on cells was studied by either transfecting Surv_Dz and Surv_mDz alone or by adding the conjugates to the cells. 12.5µM of NCL-APT-Surv_Dz and NCL-APT-Surv_mDz conjugates was added to the cells and the same concentration of APT-Linker alone was added to the cells. Cells were collected 48 h post transfection and subjected for RNA isolation and protein...
Cellular uptake of the conjugate was checked in WERI-Rb1 cells compared to the normal MIO-M1 cell line. 500nM concentration of the NCL-APT-Sur_Dz and apt-linker was used, flow cytometry analysis showed binding of the conjugate on WERI-Rb1 to be 36.62% and the apt-linker showed 37.33% to WERI-Rb1 cells. The results showed no remarkable difference thereby confirming no alteration in binding upon conjugation of aptamer upon with the DNAzyme. In the case of MIO-M1 the NCL-APT-linker was binding nonspecifically up to 14.8%, which could be due to the insertion of the linker, but upon conjugation it got reduced to 0.66%. This depicts that the conjugate is efficient in targeting cancer cells and has very less affinity towards normal cells. This remarkable property of the conjugate in targeting cancer cells alone in mixed tumor population (Fig. 2C) reflects its potentiality. Similarly, RB primary tumors were also subjected for NCL-APT-linker and APT-Sur_Dz binding. The affinity of these conjugates with tumor samples was extremely high and has very less difference between Apt-linker and APT-Sur Dz binding, as shown in the histogram the percentage uptake (Fig. 2D).

To study the delivery of the Dz to cancer cells, fluorescent Dz (Dz) was used and studied the internalization and delivery of the same. The NCL-APT-Sur_dDz chimeric conjugate internalization was imaged under fluorescent microscope. The control cell line MIO-M1 showed no binding and internalization of the conjugate or NCL-APT-linker (Supplementary Fig. 2). For studying the internalization, cells were analyzed from the surface to bottom of the cell, different Z-positions were viewed and found to observe the Dz delivery to the cells. WERI-Rb1 and Y79 cells showed internalization of the conjugate, aptamers and Dz. In the case of WERI-Rb1 cells, the NCL-APT-linker alone showed both cytoplasmic and nuclear localization, whereas the NCL-APT-Sur_dDz conjugate showed NCL-APT localized to the cytoplasmic region, while the Dz was delivered both to the nucleus and cytoplasm. The Dz alone showed binding on cell surface and feeble uptake. Thus the chimeric conjugate is efficiently internalized and delivered Dz to the cancer cells (Fig. 3). In Y79 cells too similar results were observed for the NCL-APT-Sur_dDz conjugate, while the Sur_dDz alone was able to enter into the cells. Thus there were differential mechanisms adopted by the cell lines derived from same origin. Nevertheless, the aptamer successfully delivered Dz to the cancer cells.
The functional activity of the chimeric conjugate was tested in RB cell lines, Y79 and WERI-Rb1 reported for their expression of survivin. Both the cell lines treated with APT-Dz chimeric conjugate showed significant down regulation of survivin mRNA when compared to Dz alone transfected or mDz or NCL-APT-linker and mDz chimeric conjugate (Fig. 4). The NCL-APT-linker which harbors the NCL-APT also downregulated survivin, hence the conjugate was expected to exert higher downregulation of the survivin. We also observed downregulation of survivin protein levels upon chimera treatment (supplementary Fig. 3). Decrease in survivin levels leads to apoptosis of cancer cells thereby reduces the cancer population. This cancer cell specific targeting property of the conjugate serves to be a better platform for targeting cancer cells.

Recently Sur_Dz was utilized in the MCF-7 breast cancer model and suggested possible mechanisms underlying their anticancer effects in vitro. A recent study showed, Dz against AKT1 significantly downregulated the expression in SW597 cells both at mRNA and protein levels. Our results elucidate the mechanism of action of chimeric aptamer-Dz conjugate using nucleolin aptamer and survivin Dz (NCL-APT-Sur_Dz) through participation in the proliferation or growth of RB cancer cells. Chimeric NCL-APT-Sur_Dz was shown to be more effective than survivin Dz in down-regulating the expression of survivin, which is likely a direct result of the careful design of conjugation strategy. Thus we prove our proof of concept of using chimeric aptamer Dz conjugates for targeted delivery to cancer cells. Both survivin and NCL are overexpressed in many cancer types and thus the chimeric construct can be used across various cancer types.

Conclusions

Our results demonstrated for the first time that chimeric aptamer-DNAzyme conjugate using a NCL-APT with survivin Dz(NCL-APT-Sur_Dz) could be used as a specific gene-targeting therapy to suppress progression of RB cancer as a proof of concept. This novel chimeric form may become powerful therapeutics of other cancer types in the future. Overall, down-regulating the expression and function of survivin may induce apoptosis and inhibit migration of cancer cells. Thus targeting survivin with NCL-APT-Sur_Dz is a promising and potential new therapeutic option in combating cancer.

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Notes and references


2. Nanomedicine Laboratory of Immunology and Molecular Biomedical Research (NLMBR), School of Medicine (SoM), Molecular and Medical Research (MMR) Strategic Research Centre, Faculty of Health, Deakin University, Geelong, Victoria-3217, Australia.


4. Departments of Ocular Oncology and Vitreoretina, Medical Research Foundation, Sankara Nethralaya, Chennai-600006, India.

5. Graduate student, Deakin University, Registration No. 211640938.

Electronic Supplementary Information (ESI) available: [methods and supplementary Fig. 1-3].

REFERENCES


