Review Article

Whole Cell SELEX Technology and its Applications

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Abstract | SELEX is an in vitro selection procedure to evolve single-stranded nucleic acids known as aptamers. Aptamers are known for their high selectivity and specificity and their ability to form 3D conformations. CELL SELEX is a modification of SELEX where instead of using purified ligand molecules, whole live cells are used as targets, so the aptamers being generated could bind target molecules on live cells in their native conformation and it is possible to know the molecular profile of the cellular surface. Whole CELL SELEX is very useful in the field of diagnostics and targeted therapy and the aptamers generated can be utilized to differentiate cancerous cells from normal cells or even different types of cancerous cells, detect live pathogenic organisms, and to discover novel disease biomarkers to facilitate early detection of the disease and for therapeutic purposes.

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Aptamers are synthetic single-stranded DNA or RNA molecules which have very high binding affinity and specificity for the target molecule. Also known as chemical antibodies, these short nucleotide molecules possess several unique qualities which give them additional advantages such as facile chemical modification at different sites, economical and reproducible synthesis, lack of immunogenicity, tolerance to a broad range due to physical conditions including temperature, pH or ion concentration, the conformational changes are reversible (Shieh et al., 2010). The first aptamers were generated in 1990 by two independent labs against bacteriophage T4 DNA polymerase and various organic dyes and both were unmodified RNA aptamers (Tuerk and Gold, 1990; Ellington and Szostak, 1990).

Generation of aptamers occurs by an in vitro method known as Systematic Evolution of Ligands by Exponential enrichment. Typically this process involves synthesis of combinatorial library which is composed of single-stranded DNA or RNA oligonucleotide template which contains a central random region of 20-80 nucleotides flanked by specific sequence of 18-21 nucleotides functioning as primer binding sites for PCR. This oligonucleotide library is incubated with target molecule and the portion of aptamers which are folded such that they can bind to the desired target molecule are enriched at each PCR round while those which remain unbound are washed away. The enriched pool of aptamer candidates is again allowed to bind to the target and the process is repeated. Researchers can also use negative selection methods during this repetitive process so that nonspecific molecules or the molecules common to more than one type of cells can be removed. This negative or counter selection ensures that the final pool of aptamers being selected only bind to the target cells specifically (Klussmann, 2006).

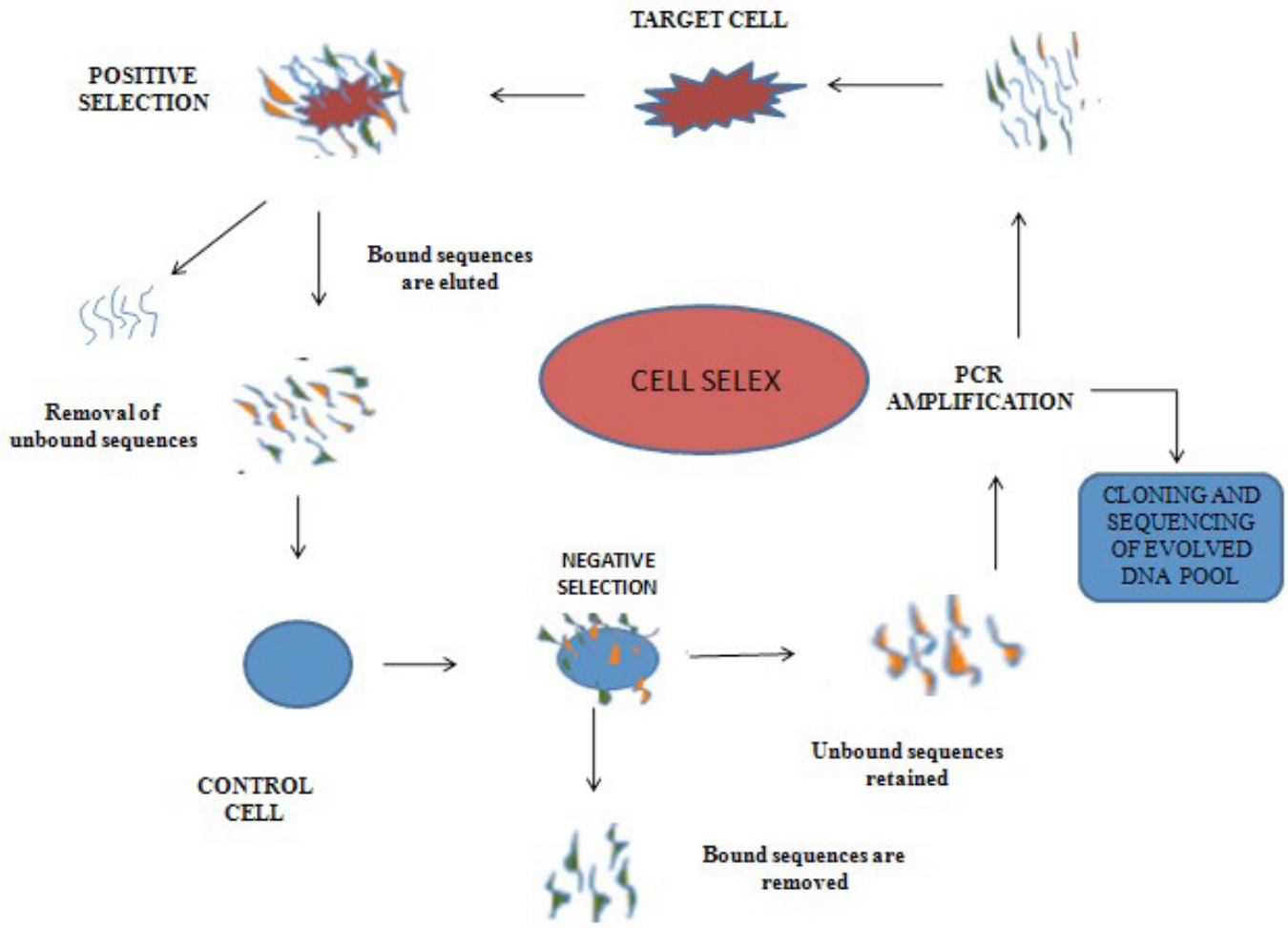

Figure 1: *A diagrammatic representation of CELL- SELEX procedure*

Cell Systematic Evolution of Ligands by Exponential (SELEX) is a modification of conventional SELEX process in which the aptamers specific to a particular cell type are produced thus making detection and capture of complex targets such as cancerous cells, virus infected cells etc. simple and straightforward. Cell SELEX offers various advantages such as discovering new biomarkers for a desired cell. As the aptamers bind the target molecule in its native state, so that one can get the whole molecular profile of a cell surface. Aptamers can be used to differentiate any two types of cells at molecular level (Figure 1).

Applications

SELEX against Tumour Cells

Aptamers which can be used to recognise a particular cell type can prove to be very significant for molecular recognition of cancer cells. By the selection and the counter selection process one can obtain aptamers which only bind to a particular type of cancer cells excluding normal or other type of cancer cells. This type of study has already been performed in which Shangguan et al. (2006) synthesized an aptamer showing affinity and specificity to human precursor T cell acute lymphoblastic leukemic cells (CCRF-CEM) and not to Ramos cells which are human B- cell line from Burkitt's lymphoma and used for counter selection (Shangguan et al., 2006). The CCRF-CEM cells were incubated with ssDNA library. The cell surface-bound sequences were eluted and incubated with excess Ramos cells. The sequences which remain unbound and free in supernatant were collected and amplified by PCR following the next round of selection. In another study aptamers specific for nasopharyngeal carcinoma cells have been synthesized (Chen et al., 2013). In this study scientists used two morphologically similar cell lines C666 cells, which are Epstein Barr (EB) virus- positive undifferentiated nasopharyngeal carcinoma cells (target) and NP 69 which is normal nasopharyngeal epithelial cell lines and used for negative screening to remove those nucleic acid molecules which bind commonly to both cell lines. A random DNA library containing 10^{15}- 10^{16} different

sequences was used. After 10 cycles of screening the DNA library was enriched. Even after the sixth cycle, the intensity of fluorescence of aptamers and C666 library stopped increasing, indicating the saturation level of binding had been reached.

Whole cell SELEX is also advantageous in cases where a signalling pathway has to be interrupted in order to prevent related cellular events that can cause cancer. One such example is of an RNA aptamer Apt-αvβ3 which has been reported for inhibition of αvβ3 integrin affecting endothelial cell growth and survival. αvβ3 integrin has a crucial role in a number of processes such as cell growth, tumor invasion, angiogenesis, metastasis and wound healing (Eliceiri and Cheresh, 2000; Brooks et al., 1995; Mitjans et al., 1995). So in this study, an RNA aptamer Apt-αvβ3 was tested for its binding ability to recombinant αvβ3 integrin (Mi et al., 2005). As per the data presented Apt-αvβ3 bound αvβ3 integrin expressed on the surface of live HUVEC. CT26 cells, which are lacking in αvβ3 integrin were used as negative control. αvβ3 aptamer was generated by in vitro transcription. Firstly, the Apt-αvβ3 was amplified from pUC19-apt- αvβ3 template using specific primers and T7 promoter sequence was incorporated via forward primer. RNA Apt- αvβ3 was transcribed from PCR generated DNA template making it resistant to RNase. Apt-αvβ3 was tested for inhibition of endothelial cell adhesion and proliferation and cell adhesion assay, proliferation assay (thymidine incorporation assay) and tube forming assay were performed on cultured HUVECs after treatment with or without platelet derived growth factor (PDGF). As per the information obtained from previous studies, it was determined that FAK-dependent signalling is involved in protecting the cells from TNF-α induced apoptosis and thus promoting cell survival (Bezzi et al., 2003). In addition, FAK activation is done by PDGF which also has an anti-apoptotic effect on cell. The results indicated that Apt-αvβ3 treated cells showed successful inhibition in cell adhesion and proliferation in both absence and presence of PDGF. In presence of PDGF, tube formation by activity of HUVEC's was also inhibited. Also it was seen that Apt-αvβ3 was capable of enhancing TNF-α induced apoptosis and down regulating the FAK signalling pathway.

One of the most concerning issue in cancer is the ability of tumour cells to metastasize i.e. to migrate and invade other normal cells. In this context, aptamers might prove to be a solution to specifically bind to tumour cells and prevent their migration as well as invasion without effecting the normal cells which also have common feature of dissemination. Already two RNA aptamers namely E37 and E10 have been synthesized and selected using isogenic cell lines of Syrian hamster embryo fibroblasts transformed in vitro by Rous sarcoma virus (Zueva et al., 2011). These cell lines differ in their metastatic potential. Actually these aptamers E37 and E10 causes changes in the phosphorylation status of various receptor tyrosine kinases thus hindering the cell signalling involved in cell migration and cell invasion (Annerén et al., 2003). Infact it was seen that on E37 binding expression of five phosphorylated tyrosine kinases namely zap70, syk, fak, Alk, Frk was diminished while aptamer E10 downregulated ErbB2 and Frk. Meanwhile when inhibitory effect of E10 and E37 was tested, it was seen that E37 could inhibit cell migration whereas E10 was effective in inhibiting both cell migration and invasion as well.

Whole Cell SELEX for Novel Biomarker Discovery

Aptamers are potential candidates for novel biomarker discovery. Since aptamers are random pools of nucleic acids so the target molecule to which the aptamers chose to bind might not be recognized earlier resulting in finding novel biomarkers. This was proven in studies by Daniels et al. (2003) and Leprini et al. (1993) in which a glioblastoma cell line, U251 was the target for SELEX by using single-stranded DNA library. The interacting nucleotides were isolated and the target protein is identified using affinity purification, liquid chromatography and mass spectral analyses (LC-MS) and ELISA. In this particular study, after 21 rounds of selection, round 12 and 21 showed increased binding to U251 cells. The round 21 pool was cloned and sequenced and after sequencing from 163 clones one aptamer GB10 based on dominance in sequenced pool and binding to U251 cells was selected. Using aforementioned biochemical strategies the target of GB10 was recognised as tenascin-C, an extracellular protein found in tumour matrix and believed to be involved in embryogenesis and oncogenesis (Daniels et al., 2003; Leprini et al., 1993).

Similarly, a DNA aptamer was generated which could be helpful in differentiating tumour micro-vessels from normal vasculature based on variation in expression of endothelial proteins. These proteins could be

indicative of diseased state and helpful in molecular profiling of cells. A transformed endothelial cell line YPEN was used as target and N9, microglial cell line was used as control. Out of 25 aptamer candidates, one aptamer III.1 was selected which was used as histological marker binding selectively to microvessels of rat brain glioblastoma but not to normal rat brain glioblastoma. The target protein of III.1 aptamer was isolated from EC and identified by MS. This protein was rat homologue of mouse pigpen (Blank et al., 2001). Pigpen is a endothelial protein of Ewing's sarcoma family involved in angiogenesis possibly highly upregulated and thus could be used as target for diagnostic and therapeutic agents (Yoshida et al., 2010).

This approach of novel biomarker discovery can be helpful in case of ovarian cancer. Ovarian cancer is the most lethal of all common gynaecologic malignancies and its diagnosis at advanced stages reduces the chances of its treatment. There is a need to identify such biomarkers which could be helpful in early diagnosis of the disease. Here as mentioned above, aptamers can prove to be really helpful in identifying novel biomarkers. In a study an aptamer TOV6 selected against TOV-21G, an ovarian cancer cell line was analysed (Van Simaeys et al., 2010; Van Simaeys et al., 2014). Its interaction with the stress induced phosphoprotein 1 (STIP 1), target on the cell membrane of the cancerous cell was investigated using mass spectrometry. Furthermore by using siRNA silencing the role of STIP involved in regulating cell invasion was established. It has also been validated that STIP-1 antibody could not bind when the gene was silenced.

Cell SELEX to Detect Live Pathogenic Organisms

Cell SELEX can also be used to identify live pathogenic organisms and can also serve a therapeutic purpose by inhibiting them. The aptamers generated against these organisms either prevent cell-cell adhesion, viral entry into the cells or antagonize or agonize receptor signalling. Cell SELEX is already been performed against *Trypanosomas brucei.* RNA aptamer binding to live parasite with high affinity have been recognised (Ulrich et al., 2002). Basically the mechanism of how the parasite escape the host immune system is because of constantly varying expression of variable surface glycoprotein (VSG) by *T. brucei* (Lorger et al., 2003). Homann & Goringer reported aptamers which can bind to the varieties of VSG variants. Initially SELEX was performed against VSG117 followed by against live trypanosomes of *T. brucei* expressing VSG117. Then following rounds of SELEX performed against VSG 221, then again followed by live parasites. The resulting aptamer showed affinities to a number of VSG variants. Aptamers have also been generated against *T. cruzi* which causes chaga disease in humans, in which aptamers compete with matrix molecule for binding to parasite to prevent parasite and host cell- matrix molecule interaction (Homann and Göringer, 1999).

Interestingly aptamer against *Bacillus anthracis* spores is reported from random synthetic 35 mer DNA library subjected to 18 rounds of selection (Zhen et al., 2002). Similarly, when talking about bacterial infections, *Mycobacterium tuberculosis* infection is a major health issue causing deaths worldwide. SELEX has already been used to obtain a new antimicrobial agent for this disease. In the research, *Mycobacterium tuberculosis* H37Rv was used as a SELEX target and *M. bovis* for counter selection. An aptamer designated NK2 was selected which dominated 30% of the final pool of 20 individual clones, selected after 10 rounds of selection. NK2 function was evaluated in mouse model which has the potential of a therapeutic drug in case of in vivo *M. tuberculosis* infection as it can increase the survival rate of mice and reduce the bacterial number in mice tissue. It was also found in the study that NK2 could improve $CD4^+$T cells to produce IFN-γ by binding to H37RV when analysis was done by infecting $CD4^+$ T cells with H37RV in presence as well as absence of aptamer NK2 (Chen et al., 2007).

Listeriosis is also a very dangerous infection which is caused by the foodborne bacterial pathogen *Listeria monocytogenes*. The mortality rate is very high (20% to 30%) among elderly and immunosuppressed individuals, so rapid and sensitive methods of detection should be developed which are easy as well as reliable. Sang-Hee Lee and colleagues developed an aptamer-based sandwich assay in which two aptamers LMCA2 and LMCA26 were used. These were selected initially out of 35 aptamer candidates. Initially the aptamers were selected as per the conventional process in which 76 base oligonucleotide ssDNA library was incubated with *L. monocytognes.* The cell bound aptamers were recovered followed by counter selection using *B. subtilis* and *E.coli* cells but the interesting aspect of this study was to use an aptamer-based sorb-

ent assay (ABSA) platform for quantitative detection. ABSA is basically comprised of three steps:

1. Immobilization of 5' amine LMCA2 on a carboxyl group activated 96 well type microtiter plate surface followed with incubation for one hour.
2. Addition of fluorescently labelled LMCA26 to individual wells.
3. Washing to remove Cy5-labeled aptamer after 30 minutes of incubation.

The *L. monocytogenes* concentration was varied ranging from 2 to $2x10^6$ CFU per ml. This ABSA platform was successful in detecting as low as the bacterial concentration of 20 CFU per ml thus providing specific, efficient and easy detection of *L. monocytogenes* (Lee et al., 2015).

In the case of viruses, aptamers have been developed against Avian influenza H9N2 virus using capillary electrophoresis based SELEX. Avian influenza virus is comprised of subtypes on the basis of surface glycoproteins and neuraminidase. Haemaglutinin (HA) is responsible for entry of virus into host cell, mediating membrane fusion between viral and target cellular membrane which can prevent antibody production. Two aptamers A9 and B4 were selected which could bind the whole virus and inhibited viral infection in host cells (Zhang et al., 2015). The aptamers effect on H9N2AIV infection was studied in Madin-Darby Canine Kidney (MDCK) cells compared with one having no aptamer, the groups incubated with A9 and B4 (aptamers) showed lower ratio of viral load to cell number. When the secondary structures of aptamers were predicted by MFOLD software, it was found that for A9 sequence double stem loop structure at site 42-61 while in B4 sequence a single large loop at site 29-42 could form G quadruplex structure. Another RNA aptamer P-30-10-16 was found that binded specifically to the HA region of target strain A/Panama/2007/1999 and did not recognize other human influenza viruses, even another strain with same subtype H3N2 (Gopinath et al., 2006).

CELL SELEX in Targeted Therapy of Diseases

Since the advent of cell-SELEX technology, targeted therapy of diseases has attained new levels. It has now become possible to deliver various therapeutics agents such as short interfering (si) RNA, drugs, toxins or nano carriers to the desired cells or tissues more efficiently. The aptamers since are easy to be chemically modified without any considerable change in its function or properties so they are functionalized with various therapeutic agents. Also the aptamers are known for their specificity and high binding affinity to the target cells, so they exclusively target the disease cells.

Aptamer-drug Conjugates

Conjugation of aptamers directly with drug moiety is relatively simple. A therapeutic agent for example the anticancer drug doxorubicin (dox) is covalently or non-covalently linked with aptamers. This functionalization of drugs with aptamers is more effective over administration of drug alone since it targets the specific tumour cells and no other tissue, thus reducing side effects which otherwise would be inevitable. This simple conjugation of aptamers and drug conjugation can be seen in cases HER2 aptamer- dox conjugates in treatment for breast cancer (Liu et al., 2012) and aptamer E_pDT3 (2' fluoro modified RNA aptamer)- Dox conjugate for treatment of retinoblastoma (Subramanian et al., 2012). In a study Chu et al. have modified PSMA aptamer with a cross linker and conjugated with recombinant gelonin using disulfide linkage. Gelonin is a ribosomal toxin that causes cell death but is unable to enter the cells efficiently. The conjugate being generated was able to enter the cancer cells with greater ease and the toxicity of conjugate was also higher (Chu et al., 2006).

We have seen till now the examples of covalent interactions but some noncovalent interactions can also be very effective in forming aptamer-drug conjugation. In a study, dox was intercalated within double-stranded region of A10 anti-prostate-specific membrane antigen (PSMA) RNA aptamer. Shieh et al. (2010) used AS1411 DNA aptamer which could form G-quadruplex structure. The G quadruplex structure is G rich and is made of four guanines by hoogsteen H-bonding. It can accommodate a quadruplex ligand 5, 10, 15, 20-tetrakis (1-methylpyridinium-4-yl) porphyrin (TMPyP4). The cationic charge on TMPyP4 stabilize the G quadruplex whereas the aptamer specifically delivered TMPyP4 into MCF7 breast cancer cells since the ligand is lacking in specificity. When irradiated by light, TMPyP4 induced photodamage in MCF7 breast cancer cells (Shieh et al., 2010).

A comparatively more efficient method of aptamer drug conjugation to enhance cellular uptake of the therapeutic agents can be seen in a study where RNA

aptamer conjugated micelles were inserted into a liposome, termed as aptamosomes and the anticancer drug doxorubicin was encapsulated in "aptamosomes". Liposome based system is advantageous in many ways as it can encapsulate even toxic or less soluble compounds and liposome surface modification can be easily done using polyethylene glycol (PEG) favouring deposition particularly at tumour sites (Baek et al., 2014).

Aptamer-Nanoparticle Conjugates

Nanoscale structures provide us a number of options which can be effectively used in imaging and therapy. Targeted therapy using nanoparticle-aptamer bioconjugates using prostate cancer as a model has been shown in a study where the therapeutic nanoparticles encapsulate docetaxel (dtxl) and is surface functionalized with A10 RNA aptamers (Farokhzad et al., 2006). The A10 2' fluoropyrimidine RNA aptamer could specifically recognize the extracellular domain of prostate specific membrane antigen (PSMA). Nanoprecipitation method was used to prepare Dtxl encapsulated, pegylated PLGA NPs (surface being functionalized with A 10 PSMA apt) (Chorny et al., 2002). These nanoparticle-aptamer biocojugate when assessed for their cytotoxicity on LNCaP cells which express PSMA protein, showed increased toxicity as compared to Dtxl-np group. This arrangement of np-aptamer bioconjugate was shown to be very specific thus reducing chances of toxicity in non-target cells and were shown to be very effective against prostate cancer. Also in vivo studies were performed to find out the efficacy of Dtxl-NP-Apt in nude mice which were injected with 3 million LNCAP cells. Complete tumour reduction was seen and survival rate was also increased as compared to those with Dtxl-NP alone and dtxl alone.

Use of therapeutics nanoparticles in targeted therapy can be advantageous in treatment of gliomas. Gliomas account for nearly 45%-50% of all primary brain tumours. Mortality rate is very high in case of gliomas (Wrensch et al., 2002; Behin et al., 2003), so in a study PEG functionalized poly (D, L-lactic-co-glycolic acid) PLGA nanoparticles were used along with an aptamer AS1411 to deliver a chemotherapeutic agent paclitaxel. AS1411 is known to bind nucleolin which is highly expressed in plasma membrane of cancer cells. Nucleolin is the target molecule for identifying C6 glioma cells and also has roles in several processes like binding and endocytosis that can be helpful in drug delivery. This arrangement of Ap-Ptx-NP has shown greater tumour inhibition in mice with C6 glioma xenografts compared to Ptx-NP alone (Guo et al., 2011).

In another study, GMT8 aptamers functionalized poly-(ethylene glycol) and poly-(ε-caprolactone) nanoparticle were used for enhanced intracellular delivery of docetaxel and increased tumour penetration using U87 glioma cells as target (Gao et al., 2012). With the advancements in the field of nanotechnology, attempts are being made to improve the drug carrying capacity of nanoparticles. Working on this idea Zheng et al. have designed a superparamagnetic iron oxide nanoparticle (SPION) based spherical nucleic acid (SNA) system through which multiple copies of aptamer

Table 1: *Other nanoparticle structures and aptamer conjugates*

Np-aptamer Conjugates	Remarks	References
Gold nanoparticle (AuNP)-EGFR RNA aptamer	Nonspecific absorption of AuNP's reduced.	Li et al. (2010)
AuNP-sgc8c DNA aptamer-hairpin DNA	Repeated sequence within hairpin DNA to load Dox.	Luo et al. (2011)
AuNR (Gold nanorods)-sgc8c DNA aptamer	3' end of aptamer linked with chlorine e6 (a photosensitizing molecule). The AuNR-apt conjugate is used for photothermal therapy.	Wand et al. (2012)
Hollow gold nanospheres (to carry dox)-RNA aptamer	RNA aptamer targeted CD30 a biomarker for Hodgkin's lymphoma	Zhao et al. (2013)
Single-walled carbon nanotubes (SWNTs)-sgc8c DNA aptamer daunorubicin	Sgc8c DNA aptamer targeted PTK7, a leukemia biomarker protein. SWNTs enhanced targeted delivery of daunorubicin to Molt-4cells (acute lymphoblastic leukemia T cell line).	Taghdisi et al. (2011)
Quantum dots- MUC-1 aptamer dox conjugate. Dox and quantum dots are connected by hydrazone bond that resulted in release of drugs in endosomes	Quantum dots-MUC-1-Dox conjugate were specific for ovarian tumours and showed increased toxicity vs. free Dox.	Savla et al. (2011)

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AS1411 can bind on the surface of SPION by hybridizing to the DNA biopolymer (Zheng et al., 2013). Another group of magnetic nanoparticles named as porous hollow magnetic nanoparticles (PHMNPs) are being used for targeted therapy of cancer cells. The porous structure is meant for drug loading and this porous shell is stable under basic and neutral conditions.

Chen et al. (2011) have functionalized these PHMNPs with PEG having active carboxyl groups and aptamers with amino groups were crosslinked via N-hydroxysuccinimide on the surface of PHMNPs. Dox, the anticancer drug is loaded in the porous shell and the apt-PHMNPs on entering the targeted CEM cells is inhabited in the lysosomes. The acidic environment in the lysosomes results in the release of the drug and killing the target cells (Chen et al., 2011) (Table 1).

Aptamer-siRNA Conjugates

Aptamers are also being used for cell type specific delivery of siRNAs taking advantage of the phenomenon RNA interference to silence the desired genes. RNA interference is a naturally occurring process that is used by cell to turn off or silencing of genes in order to stop the synthesis of respective protein and also as a defence mechanism against viruses. RNA interference is triggered by double-stranded RNA that binds the DICER protein and cleaved into smaller fragment. In a study James O McNamara and colleagues developed A10 aptamer-siRNA to PSMA expressing cells (McNamara et al., 2006). The siRNA that was delivered targeted PLK1 and BCL2, two survival genes expressed in tumour cells. It was seen that these A10 chimeras do not trigger interferon response thus preventing any nonspecific inflammatory response. Also A10 –PLK1 chimera in addition was found to promote tumour regression. Similarly, HER-2 aptamer and BCL-2-siRNA chimeras have been developed to silence BCL-2 expression as a repercussion of which the HER-2 positive cells became sensitive to low dose chemotherapy.

Zhou et al. (2013) used BAFF receptor aptamers to deliver siRNAs to non-Hodgkin's lymphoma cells. The B cell activating factor (BAFF) receptor is overexpressed in non- Hodgkin's lymphoma and when the ligand BAFF binds to the receptor, there is an increased cell proliferation and the expression of BAFF is controlled by STAT-1 and STAT-3 signalling pathway. So an aptamer-siRNA chimeras were developed to specifically knockdown expression of STAT-3 (Zhou et al., 2013).

An interesting approach is applied for targeting HIV-1 tat/rev common exon. An anti-HIV-1-gp120 aptamer was covalently linked to siRNA and both the aptamer and siRNA have anti HIV activities. Gene silencing was observed when the aptamer-siRNA chimera was internalized into HIV-1 infected CEM-T cells. When the efficiency of the aptamer-siRNA chimera was tested in vivo in HIV-1 infected humanized Rag-Hu mouse model it was seen that the chimera inhibited HIV-1 replication and normal complement of $CD4^{+}$T cells were found which otherwise would be depleted by viral infection (Zhou et al., 2008; Zhou et al., 2009). Not only siRNA but siRNA and miRNA are also being used to form aptamer-RNA chimera against targeted cells like in case of prostate cancer cells a siRNA against DNA dependent protein kinase gene (DNAPK) was fused with anti PSMA A10RNA aptamer and thus reduced the expression of DNAPK in prostate cancer cells expressing PSMA (Raval et al., 2013). Liu et al. (2012) have primarily reported the use of MUC-1 aptamer (against mucin1) for tissue specific delivery of let-7i miRNA into OVCAR-3 ovarian cancer cells which after cleavage by DICER sensitized the cells to the treatment of paclitaxel (Liu et al., 2012). Mucin 1 which is a cell surface glycoprotein in cases of human adenocarcinomas is found to be overexpressed and glycosylated (Altschuler et al., 2000). As a result inhibition of cell proliferation was observed alongwith induced cell apoptosis.

Future Prospects and Challenges

Cell SELEX has great deal of potential when it comes to diagnostics or targeted therapy. With the advances in other fields like nanotechnology, proteomics and imaging techniques, aptamers generated through cell SELEX are offering very promising alternatives to antibodies due to their property of target specificity and selectivity and their capacity to form 3D structures. But it is very important to bridge the gap between theory and practical applicability of the process so that this technology can be put into effect for clinical applications. Although aptamers offer a great deal of advantages over antibodies such as lack of immunogenicity, ease of chemical modifications among others but there are still limited number of clinical aptamers in use. Cell- SELEX has proven to be very effective in vitro but its in vivo applicability is still un-

der consideration. RNA aptamers are considered to form strong interactions and thus capable of forming more stable 3D structures but stability of RNA has always been an issue. The modifications although can stabilise RNA but comes at a very high cost. Also the chemical oligonucleotide synthesis technology currently in use has some yield limitations when it comes to longer aptamers or to form conjugates of aptamers with the therapeutic agents (Bruno et al., 2013; Bruno et al., 2008).

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