Apta2R: Aptamer Selection for Vesicular Stomatitis Virus Receptors on Vero Cells

Honours Thesis Project for the Faculty of Science at the University of Ottawa for partial fulfillment of the Bachelor of Science Degree

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Acknowledgments

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Most of all, I would like to thank Dr. Maxim Berezovski for his guidance, and for giving me the opportunity to work in his lab and be a part of this important research.

Abstract

Aptamers, short-singled stranded DNA sequences, can bind specifically to different target molecules via electrostatic interactions. Their specific binding affinity, with the capability of modification, is very appealing for targeting specific and even unknown biomarkers of diseased cells. The aim of this project is to select aptamers for Vero cells using a Systematic Evolution of Ligands by Exponential Enrichment (cell-SELEX) approach termed Aptamer-to-Receptor (Apta2R). Aptamers are incubated with cells then aptamers are competitively displaced from their binding sites on the cells by adding vesicular stomatitis virus (VSV). The aptamers selected will be an indicator of where VSV binds for infection and killing of interferon-deficient cells. The aptamers are therefore targeting an unknown biomarker of these cells. The aptamers are also being negatively selected against red blood cells (RBCs) to reduce non-specific aptamer binding.

These aptamers could be used as diagnostic tools for cancerous cells as well as a potential specific drug delivery method for cancer treatment. Compared to antibodies, which are commonly used to treat disease, aptamers are more stable, selective, and cost efficient. Evidence of positive binding of the selected aptamers to interferon-deficient or cancerous cells also means that oncolytic virus therapy would be another possible treatment strategy.

Aptamer pools were successfully selected in this project and show positive binding to the target Vero cells. Continued flow cytometry studies will be able to determine the binding affinities of these aptamer pools and provide further evidence to confirm their binding to the same receptor as VSV.

Introduction

Finding a molecular probe for biomedical applications can be a very difficult task to accomplish in today's world of science and disease. Aptamers, first used in scientific studies in 1990, are an up and coming solution to this problem [4]. Aptamers are short single-stranded oligonucleotides usually 20 to 100 nucleotides in length. Individual aptamers can form unique 3D structures under certain conditions including stem loops and other complex structures involving hydrogen bonding. When the aptamers are in their 3D forms, usually around room or body temperature, they can form non-covalent bonds with a specific target [4].

Aptamers can be applied to a wide variety of biomedical applications including selection to small molecules or proteins as well as diagnosis of diseased cells and specific drug delivery [3,7]. Aptamers can be used as an indicative tool of a certain type of target by using fluorescent labels that become activated when the aptamers bind to their target. Aptamers can also be conjugated to other molecules, including drugs, which could be delivered directly to the target upon binding.

Aptamers are also rumoured to be the new alternative to antibodies since aptamers can bind specifically to a target and have a certain effect on the target when bound. They have many critical advantages over antibodies including low immunogenicity, low toxicity, increased selectivity and specificity for targets even in complex mixtures [10]. Aptamers are also easy to synthesize, can be degraded in the body by endonucleases and excreted by renal clearance without any known adverse side effects [4].

Cancer treatments such as chemotherapy, radiation, and other drugs have many adverse side effects that also affect healthy cells in the body. Vincristine, for example, is a drug commonly used to treat cancer and it acts by inhibiting the mitotic spindle in cells [11]. Inhibiting the mitotic spindle in a cell stops the chromatids from being separated during cell division and this consequently halts cell division. Without a specific drug delivery mechanism to cancerous cells, this drug acts on all cells of the body and inhibits cell division of healthy cells. This is why patients being treated with drugs like vincristine and other forms of chemotherapy often lose their hair [11]. Hair follicles are fast dividing cells that require mitotic spindle to divide. These patients can also suffer from low white blood cell counts which can be dangerous for the immune system and general health.

Discovering biomarkers for diseased cells is key since researchers aim to selectively correct or kill diseased cells and leave healthy ones unaffected by the treatment. Finding biomarkers is not an easy task and may require many experiments, complex approaches, as well as expensive equipment. Some conventional methods for the discovery of biomarkers can include mRNA screening, 2D-gel electrophoresis and mass spectrometry [2]. The difficulties with these approaches are that they all require cellular disruption prior to use so the biomarkers cannot be discovered in their standard states [2]. For example, the localization or other interactions the biomarker has in the cell could be affected by cellular destruction and therefore be an inaccurate indicator of a biomarker [2]. Most of these conventional methods are prone to false positive and false negative results as well which are not useful and could be a huge disadvantage for the biomarker discovery processes [2].

Very few biomarkers are available for effective cancer diagnosis and this is where aptamers could provide an inexpensive, efficient, and selective way to diagnosis and treat cancer [10]. Aptamers, in most cases, bind to the exterior of cell membranes and could be a useful tool for finding a biomarker on the cell membrane; for example, a protein expressed only on cancerous cells. Another great quality of aptamers is their ability to bind to a target that may not be known or characterized at this point in time. The biomarker can subsequently be analyzed and discovered after the aptamer selection process so after an aptamer probe has been developed for the target.

SELEX (Systematic Evolution of Ligands by Exponential Enrichment) is a fast and efficient method to generate aptamers that bind specifically to a target. In this project, a modified cell-SELEX approach is used to select aptamers to receptors on Vero cells. Selecting aptamers to an unknown receptor is also termed Apta2R by the Berezovski Research Group meaning Aptamer-to-Receptor, or aptamer selection to a target receptor. Cell-SELEX involves separating DNA (aptamer) that is bound to the target from the free DNA that did not bind to the target. The next step is isolation and amplification of the bound DNA using the polymerase chain reaction (PCR) and repeating this cycle 10-20 times using the enriched aptamer pool for the proceeding round of selection. The single stranded aptamers generated for the next selection round must be separated from the complementary DNA after PCR. This can be done using asymmetric PCR which is used in this project and is quite efficient. The asymmetric PCR mixture contains a 20:1 ratio of forward primer to reverse primer in order to generate a larger amount of the desired aptamer sequence and minimal amounts of complementary aptamer or random aptamer sequences.

Negative selection of aptamers can also be performed to ensure that the aptamers selected bind only to the desired target[1]. It is important to do negative selection analysis to ensure that the aptamers selected are actually for a biomarker indicating disease. Negative selection is usually applied to healthy cells such as red blood cells or bone marrow aspirates [10].

After aptamer selection, the aptamers can be cloned into bacteria and sequenced. Different aptamer sequences can be compared and even optimized to obtain aptamers with the best binding affinity for their desired use. Aptamers can be further optimized by making nucleotide changes to either increase binding specificity or affinity to the target or decreasing the possibility of the aptamers binding to non-targets.

Unfortunately, there is no aptamer targeted for cancer diagnosis or treatment at this time. There is currently only one aptamer being used that has been approved by the Food and Drug Administration (FDA) in 2004 [6]. Pegabtinib, also known by the brand name Macugen, is currently used to treat age related macular disease also known as AMD [5]. It is caused by the increased production of blood vessels and their leakage in the eye [5]. Left untreated, AMD can result in blindness. The aptamer treatment is targeted to the vascular endothelial growth factor 60 protein to inhibit the production of new blood vessels [5]. The aptamer can be injected locally to the eye so it undergoes minimal degradation and can reach its target VEGF-60 quickly and prevent blindness [5]. This type of treatment strategy gives aptamers hope for new and more difficult applications in the future.

This honours research project aims to explore the ability of aptamers in binding specifically to Vero cells, a cell type that has a diminished interferon pathway. An interferon pathway in cells, when activated, initiates an immune response that is responsible for triggering an anti-viral response [8]. Most cancer cell lines also tend to have a reduced interferon pathway and this leads to easy viral infection [8]. This is why research is leaning towards an oncolytic virus based approach to treat cancerous cells. Oncolytic viruses can easily and efficiently infect cancer cells lacking an interferon pathway and trigger apoptosis or cell death [8]. The advantage of using oncolytic viruses for this application is that normal or healthy cells are not targeted or infected since they are capable of the proper interferon response. The vesicular stomatitis virus (VSV) is a known oncolytic virus capable of infecting cancerous cells and is used as a competitive binder to displace aptamers from their binding sites on the Vero cells. A Vero cell line can be grown continuously in cell culture and can also form tumours because they are abnormal cells [6]. Vero cells are extremely sensitive to viral infection and are commonly used for studying and propagating viruses especially due to their diminished interferon pathway that they share with some other cancer cell lines [6]. Experimenting with Vero cells and VSV could lead to the discovery of a promising aptamer for use in cancer diagnosis and treatment.

It is thought that aptamers bind selectively to specific proteins located on the exterior of cells [10]. These proteins are a target for the selected aptamers and the receptor can be characterized by mass spectrometry which would be an interesting next step in studying diseased cell receptors and determining what these proteins are as well as why and how they differ from healthy cells.

Since the aptamers in this project are being competitively displaced from their binding sites by VSV, this ensures that the aptamers being selected are binding to the cells on the same cell receptors that the virus is binding to. With further research, these binding sites could be characterized using mass spectrometry and other more in depth analyses.

So, using VSV and/or aptamers as a cancer treatment strategy is a promising approach since it has fewer side effects and is more selective compared to standard cancer treatments such as chemotherapy, radiation and other drugs as discussed above.

The aptamers selected in this experiment could be tested by removing a tumour from a cancer patient and detecting if the aptamers bind to the cells by fluorescence analysis. If there is binding, we could potentially use oncolytic viruses as a treatment strategy for these patients which is currently being explored by Dr. Maxim Berezovski's Research Group at the University of Ottawa among others worldwide [8].

Aptamers are selected using the modified cell-SELEX approach as shown in Figure 1. This approach is commonly used in selection of aptamers for cells especially in the Berezovski Research Group Lab and is becoming increasingly popular in other labs across the world due to its efficiency in selecting aptamers and the accurate results obtained. The technique being developed in the Berezovski Research Group for discovery of biomarkers using aptamers is termed AptaBiD, Aptamer-Facilitated Biomarker Discovery [1].

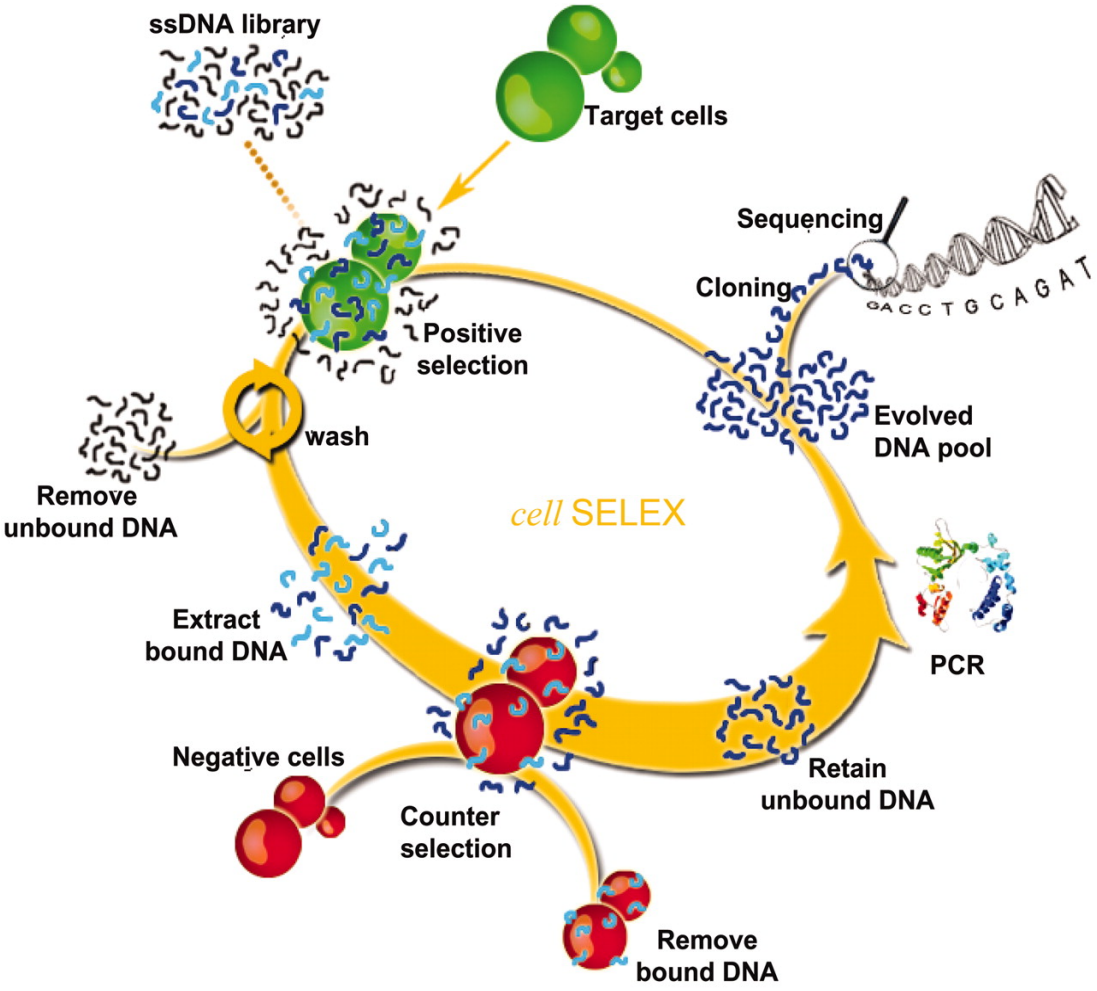

Figure 1. A schematic showing the overall steps of cell-SELEX, selecting aptamers for a target cell and consequent counter selection against non-targets. Cloning and sequencing to follow selection. Photo from: http://www.chem.ufl.edu/~tan/group/chemicalbiology.html

Aptamers are a promising tool for the recognition and diagnosis of disease since they can be applied to a wide range of targets including cells. If these tools could eventually be applied for use in patients suffering from disease, early and efficient recognition could increase patients' chances of survival and increase the effectiveness of drug delivery [3].

The aim of this project is to select and further characterize aptamers that bind selectively to Vero cells and obtain these aptamers by using VSV to displace the aptamers bound to the cells. This report emphasizes the experimental procedure, results, and discussion of these results as well as a conclusion and ideas for future research ideas in the same area of study.

Materials and Methods

Cell Culture:

Vero cell line (CCL-81) was obtained from ATCC. Cells were cultured using Dulbecco's Modified Eagle's Medium (DMEM) (Sigma, #5546) with 10% Fetal Bovine Serum (FBS) (Sigma,#F2442). Dulbecco's Phosphate Buffered Saline (Sigma,#8662) was used for washing the cells and Trypsin-EDTA (Sigma, #T4049) was used to detach cells from culture plates. All experiments involving cells and aptamers were performed in a Canadian Cabinets Biohood. Cells were stored in a ThermoScientific HERAcell 150i CO_2 incubator at 37˚C.

A synthetic aptamer library was used at the beginning of the first round of selection (Integrated DNA Technologies, USA). Each aptamer sequence contains 2 primer sites (forward and reverse) flanking the 40 base random region (5' - CTC CTC TGA CTG TAA CCA CG – 40 random nucleotides - GC ATA GGT AGT CCA GAA GCC - 3'). The aptamers obtained after each round of selection were amplified using symmetric followed by asymmetric polymerase chain reaction (PCR) and used in subsequent rounds of selection. Each pool was denatured at 95˚ C for 10 minutes then placed on ice for 10 minutes prior to the next round of selection.

Positive aptamer selection protocol:

100 nM library aptamer pool was applied to 1 well of Vero cells (100 uL) ensuring complete coverage of the cells. The cells and aptamers were incubated for 30 minutes in a 5% CO_2 incubator at 37° C. The well of cells and aptamers were then washed two times for rounds 1 through 5 and three times for rounds 6 and 7 with 1X warm DMEM to remove unbound aptamers. 1.6×10^{10} PFU of vesicular stomatitis virus (VSV) in DPBS (total of 60 uL) was added to the cells and incubated for 45 minutes in the 5% CO_2 incubator. The medium was then

collected for symmetric and asymmetric PCR amplifications followed by purification before being used in the next round.

Negative aptamer selection protocol:

Plasma-free human blood was used for round 8 and plasma-free mouse blood was used for round 9 of selection. 50 uL of blood was mixed with 50 uL of PBS and this sample was centrifuged for 10 minutes, 3000 RCF at 16° C. The supernatant containing blood serum was discarded.100 nM of the previous aptamer pool in DMEM was added to the pellet containing red blood cells and platelets and was then incubated for 30 minutes in the 5% CO_2 incubator at 37° C. The sample was centrifuged for 10 minutes, 3000 RCF at 16° C and the supernatant was added to 1 well of Vero cells then incubated for 30 minutes in the CO_2 incubator at 37° C. The cells were removed from the well by adding 40-50 uL of trypsin followed by 4X DMEM serum-free media. The well was washed with DPBS then the sample was centrifuged for 10 minutes, 3000 RCF at 16° C. The supernatant was discarded and 40 uL of DPBS was added to the pellet and was heated for 10 minutes at 95° C. The sample was then spun down at the highest speed possible (72,000 RCF) for 10 minutes and the supernatant was amplified first symmetrically then asymmetrically by PCR.

The symmetric and asymmetric PCR protocol:

The symmetric and asymmetric PCR master mixes contained 1X Buffer (an equal combination of Buffer A, B, 2A and Enhancer from Kapa Biosystems). The asymmetric master mix also had 0.1X GreenGoTaqFlexiBuffer (Promega) mixed with buffer. The master mixes also contained 1.1 mM $MgCl_2$ (Promega), 220 uM dNTPs, 11 U Kapa 2G Robust HotStart Polymerase (Kapa Biosystems) and nuclease free water to a total volume of 1500 uL. The

symmetric master mix contained 0.33 uM of both F4 forward primer and R4 reverse primer. The asymmetric master mix contained 1.1 uM of F4 primer and 5.5 x 10^{-2} uM of R4 primer. Thus, symmetric master mix used an equal amount of forward and reverse primer whereas the asymmetric PCR amplification used approximately a 20:1 ratio of forward primer to reverse primer. The F4 forward primer (5' - CTC CTC TGA CTG TAA CCA CG - 3') and R4 reverse primer (5'- GGC TTC TGG ACT ACC TAT GC - 3') were used to amplify the enriched aptamer pools after selection. Both PCR master mixes were aliquoted into PCR tubes, each sample being 45 uL.

After the medium was collected from either positive or negative selection round, 5 uL was added to a symmetric PCR tube containing 45 uL of master mix and amplified by PCR. 5 uL from the symmetric sample was added to the 45 uL asymmetric samples and amplified again by PCR. The PCR protocol for both symmetric and asymmetric amplification was initial denaturation at 95° C for 2 minutes, followed by 15 cycles of 95° C for 30 seconds, 56.3° C for 15 seconds and 72° C for 15 seconds, then held at 4° C.

Aptamer purification and gel electrophoresis:

Pall centrifugal devices Nanosep 30K (Omega) were used to purify the aptamer pools. 80 uL of asymmetric aptamers were centrifuged for 13 minutes, 3800 RCF at 16° C. The aptamers were washed with 80 uL DMEM then centrifuged for 10 minutes, 3800 RCF at 16° C. Another 80 uL of DMEM was added to the aptamers and the samples were centrifuged for 5 minutes, 3800 RCF at 16° C. 20 uL of Dulbecco's Phosphate Buffered Saline (DPBS) was

added to the filter and left for 5 minutes,aptamers were then collected for gel electrophoresis to check for products.

The asymmetric and purified aptamer pools were run on a 3% agar gel using 1X TAE buffer. The gels were then analyzed using Alpha Innotech Imaging System (Fluoro Chem Q). Once an aptamer product was verified, 100 nM of the sample could be used in the next selection round.

Flow Cytometry protocol:

All aptamer pools were analyzed using flow cytometry on an FC-500 flow cytometer (Beckman Coulter, Inc. USA). 100 nM concentration of all pools was prepared. Vero cells were counted using a Petroff-Hausser Counter from Hausser Scientific for counting sperm and bacteria. One million cells were initially used in each sample of aptamer pools. 100 nM of purified aptamer was added to each sample and all samples were incubated for 30 minutes in the CO_2 incubator at 37° C. The samples were then split in half, half of the cells had 10 uL of VSV added (1.6×10^{10} PFU) and the other half had 10 uL of PBS added. The samples were added to flow tubes (with approximately 500,000 cells per tube) and DMEM was added to a final volume of 350 uL. All of the samples were then run through the flow cytometer. All results were analyzed by Kaluza Analysis Software (Beckman Coulter, Inc. USA).

Results and Discussion

Seven rounds of positive selection were performed successfully to the Vero cells. The main steps of aptamer selection involved applying the initial synthetic aptamer library to the cells, incubating and washing with DMEM. The vesicular stomatitis virus (VSV) was added to displace aptamers that bound to the same membrane receptor on the Vero cells as VSV. The aptamers that were displaced in solution were collected and amplified by PCR for further analysis. A schematic showing the aptamer selection process specific to this experiment can be seen in Figure 1. The aptamer pools were analyzed by gel electrophoresis to confirm the presence of a product after each round and an example of a gel that was run after purification can be visualized in Figure 2. There are two bands in each lane where the aptamer pools were run. The upper bands correspond to a larger molecular weight (~80 nucleotides) which is the aptamer product from selection whereas the lower bands correspond to a smaller molecular weight (~20 nucleotides) which is remaining primer DNA that was not removed during purification. Further purifications could be performed to remove primer contamination. After analyzing the gel using Alphaview, 100 nM of each round was prepared to be used in the next round.

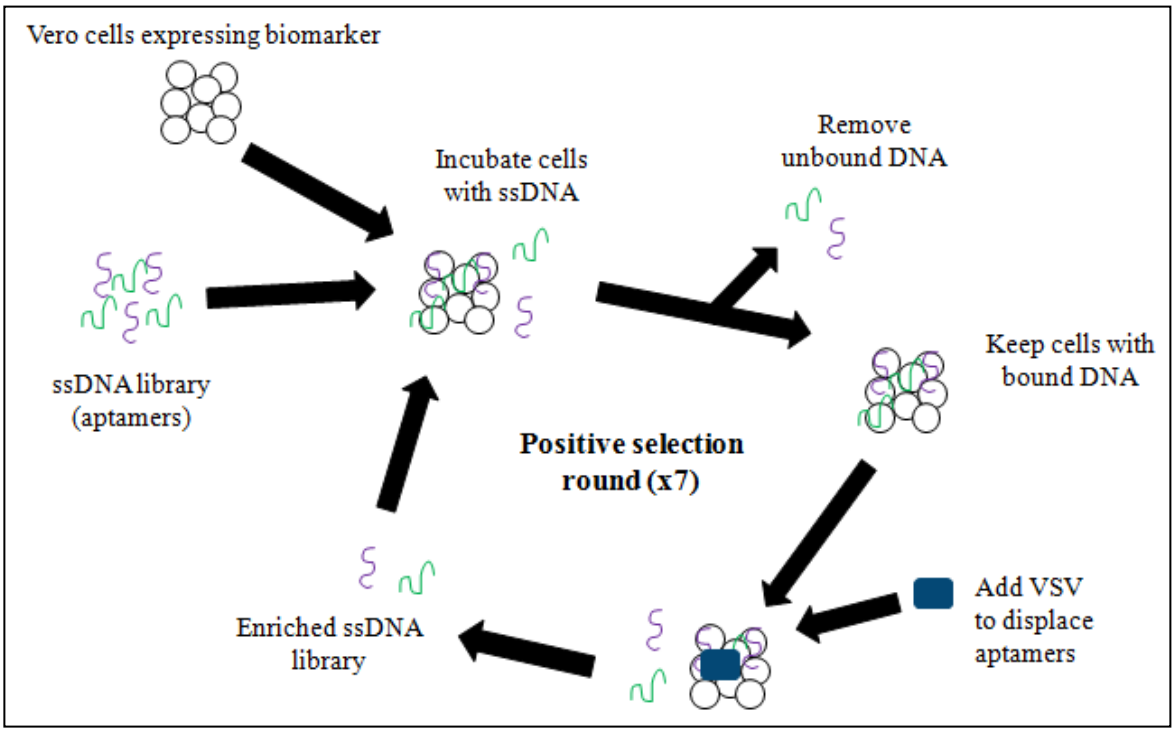

Figure 1.A schematic showing the main steps of the cell-SELEX positive selection round. VSV is added to the Vero cells after incubation with aptamer in order to displace the aptamers that bind to the same location or receptor on the cell membrane as VSV. The enriched ssDNA library is amplified symmetrically then asymmetrically by PCR before being used in the next selection round.

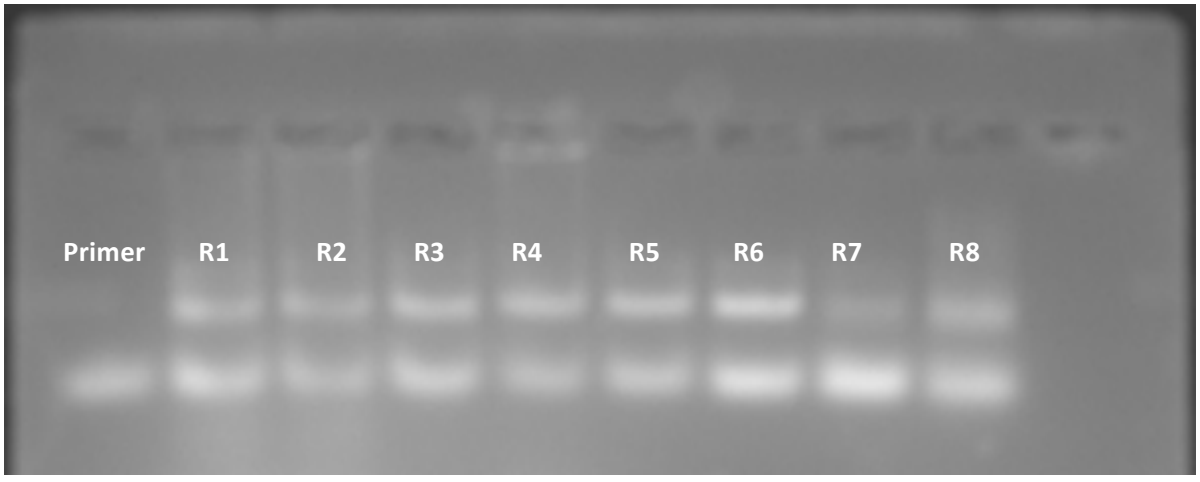

Figure 2. Gel electrophoresis showing the purified products of rounds 1 through 8 of aptamer selection. The control used to indicate the size of the products is in lane 1, the 20 nucleotide long primer F4.

All aptamer pools were analyzed by flow cytometry. The Vero cell population is shown in black for the Kaluza Figures 3, 4, 6 and 9. This population was not normalized before analysis but the results shown are representative of the fluorescence changes obtained and discussed.

From Figure 3, we can see that the different pools have varying fluorescence intensity when bound to the target cells. This figure only shows fluorescence intensity of some different aptamer pools after being incubated with aptamer for approximately 1 hour. The results show that for each successive round of selection, the fluorescence intensity decreases slightly. This could mean that as the aptamers are being selected to their specific target on the cell membrane surface these specific receptors are becoming saturated with aptamer. The first few rounds may contain a lot of non-specific or weak binding aptamers that would be removed in successive rounds. This would be explained by an increased number and length of medium washing steps during later rounds of selection.

All aptamer pools did show an increased fluorescence signal compared to the Vero cell population. Of all aptamer pools, round one of aptamer selection shows the highest fluorescence signal suggesting this pool has the highest percentage of aptamer bound to cells. It is important to note that after one round of selection there could still be a lot of weak binding or non-specific aptamers binding to the cells causing an increase in the fluorescence intensity signal. This hypothesis is further confirmed by Figure 4 which shows that the initial aptamer library has a high level of fluorescence, even more than round one aptamer pool when compared to the Vero

cells. The aptamerlibrary contains an exponential amount of aptamers that could be binding to any part of the Vero cell membranes and may not be removed during initial washes.

For each consecutive aptamer pool after round one, there was an overall negative shift in fluorescence signal. A possible reason for decreased fluorescence is that as aptamers for the target receptor are being selected, there is saturation of these receptors and elimination of aptamers that are not binding specifically to these cells or that are binding weakly as mentioned before. This would explain why aptamer pool nine consistently shows less fluorescence than aptamer pool one.

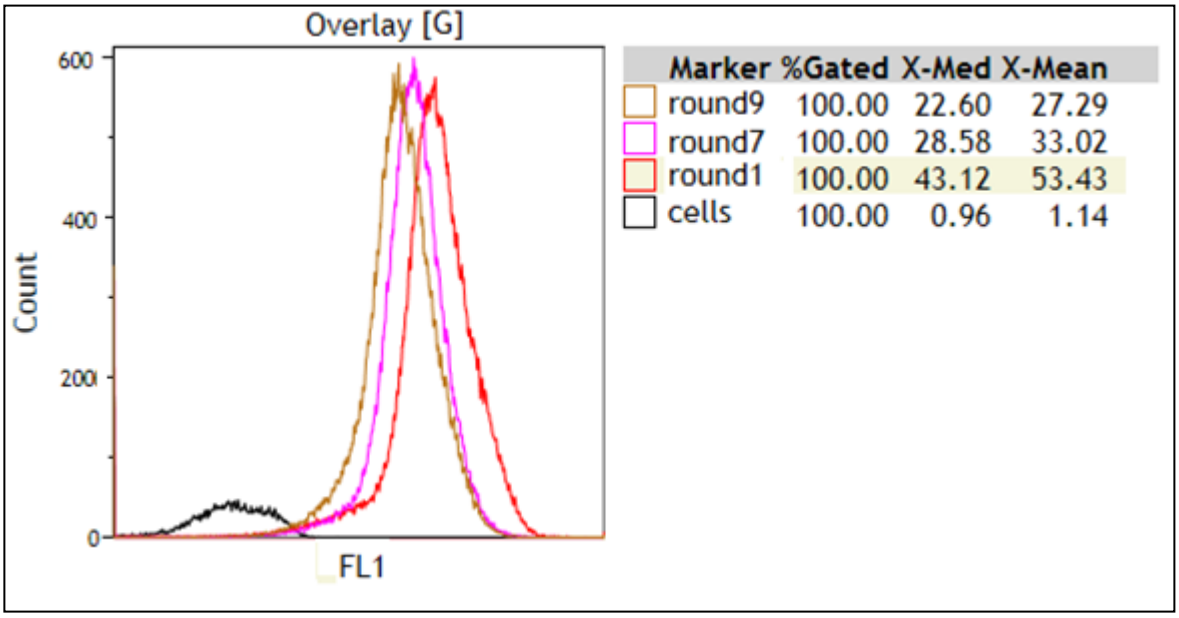

Figure 3. Flow cytometry overlay showing the measurement of fluorescence intensity of Vero cells incubated with aptamer pools 1, 7 and 9 compared to the Vero cell control. The cell count shows the number of cells analyzed.

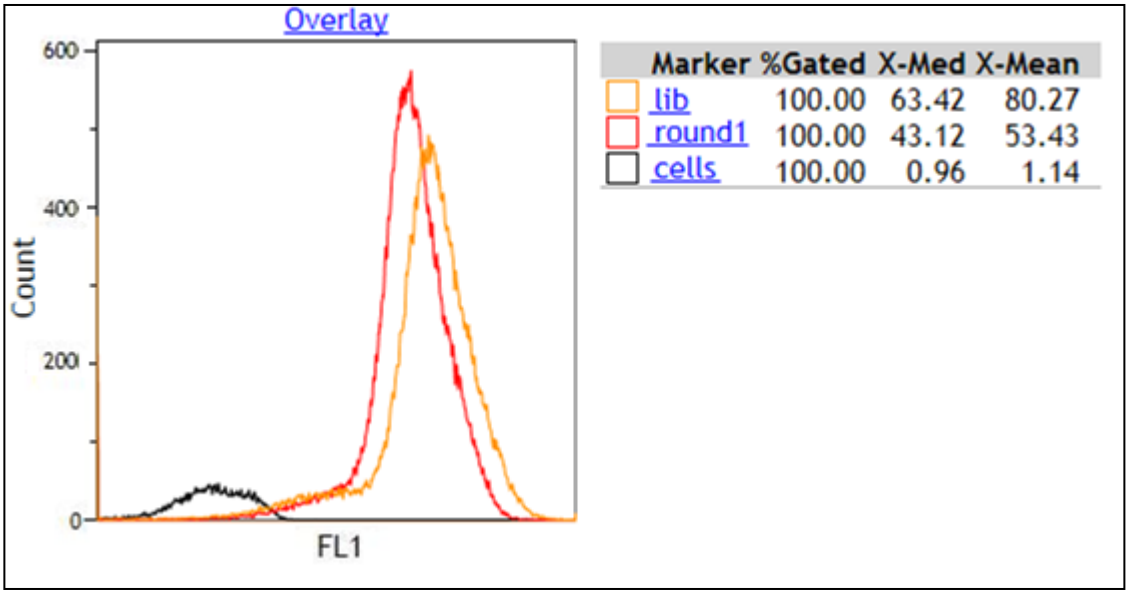

Figure 4. Flow cytometry overlay showing the measurement of fluorescence intensity of Vero cells incubated with the initial DNA aptamer library, and aptamer pool 1 compared to the Vero cell control. The cell count shows the number of cells analyzed.

Two rounds of negative selection were performed, one to human red blood cells and the other to mouse red blood cells. The schematic showing negative selection can be seen in Figure 5. The enriched ssDNA aptamer pool from positive selection is first added to red blood cells (RBCs) and incubated, the unbound DNA is taken and added to the Vero cells. This is to ensure that aptamers being selected are specific to Vero cells and that they are not binding to a receptor common to both cell types. The Vero cells and aptamer complexesobtained are heated to displace the aptamers from their binding sites then they are collected for PCR amplification.

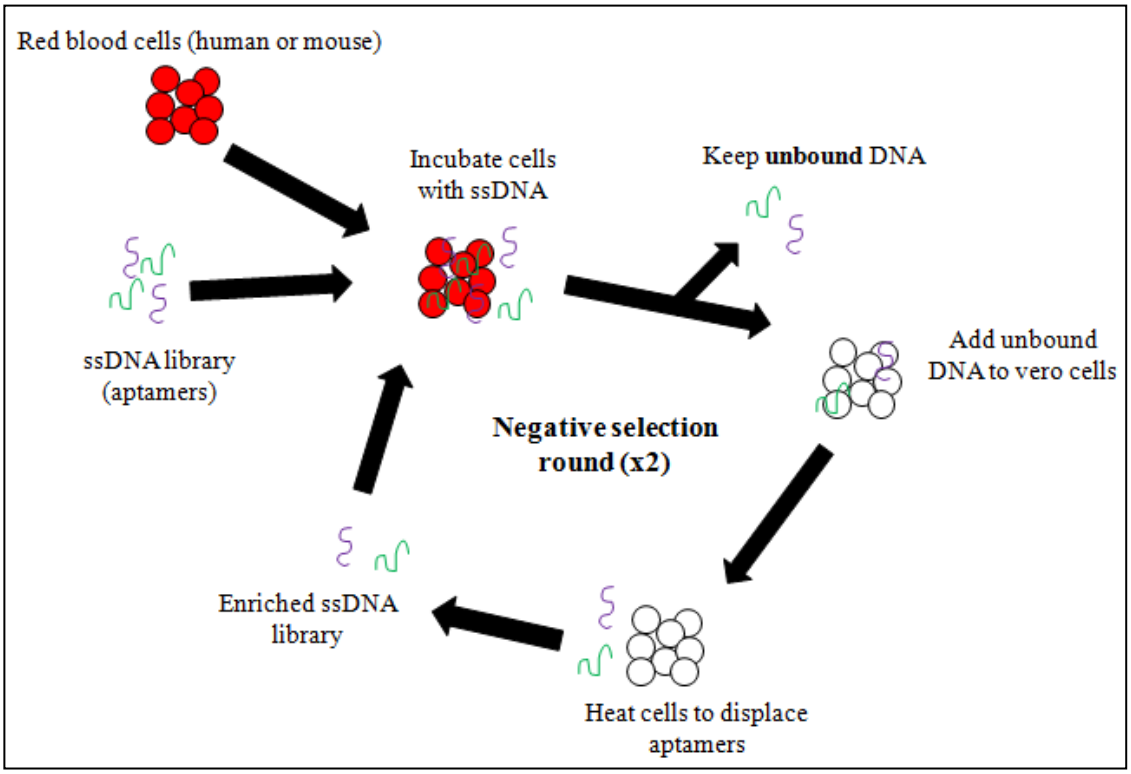

Figure 5.A schematic showing an overview of cell-SELEX negative selection to plasma-free red blood cells. The enriched ssDNA library is amplified symmetrically then asymmetrically by PCR before being used in the next selection round.

During the third round of negative selection (the 10th round of selection overall), no product was visibly detectable on the gel after three attempts at re-doing the round. It is hypothesized that perhaps there was a high amount of non-specific binding of aptamers and/or aptamers with low affinity to the target Vero cells and that the negative selection protocol should be modified[1]. It is also possible that no aptamer product was obtained from this round. All negative selection rounds should be re-done if this is the case. A potential solution to this problem would be to add masking DNA, yeast RNA for example, to the pellet of red blood cells before adding the previous pool of aptamers[1]. The masking DNA could cover non-specific binding sites and an increased amount of specific aptamers could be collected and amplified then a product would be visible on the gel[1].

Rounds 8 and 9 of aptamer selection (the only two negative rounds successfully performed) were analyzed by flow cytometry and showed decreasing fluorescence signals compared to the first aptamer pool (Figure 6). There was most likely a decreased amount of aptamers selected for the specific target which lead to no detection of aptamers during gel electrophoresis as mentioned above.

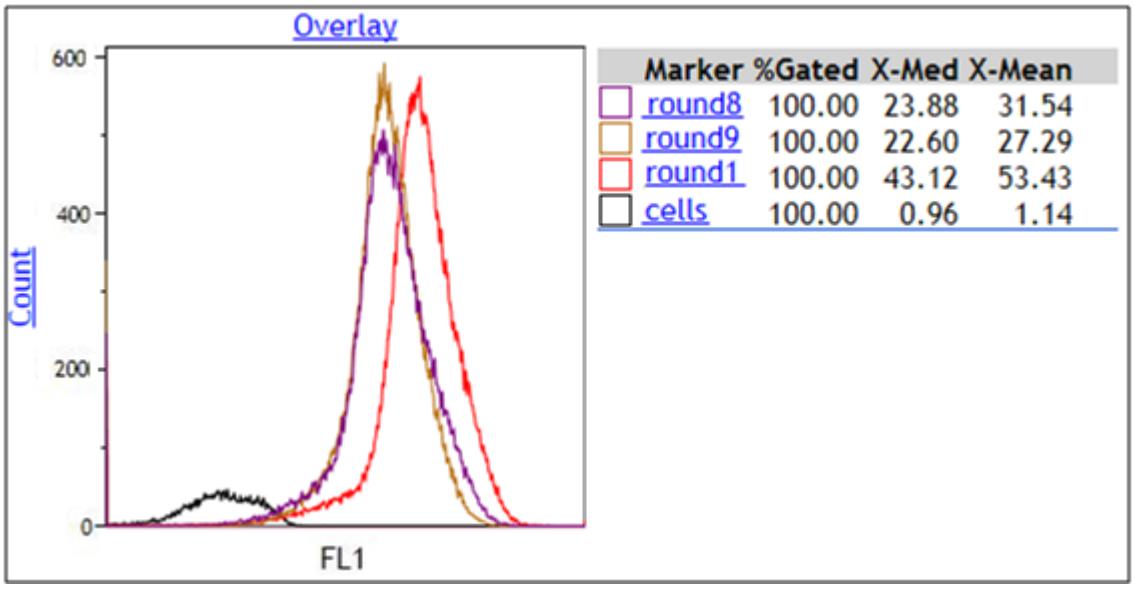

Figure 6. Flow cytometry overlay showing the measurement of fluorescence intensity of Vero cells incubated with aptamer pools 1, 8 and 9 compared to the Vero cell control. The cell count shows the number of cells analyzed.

The flow cytometry results from Figure 7 show that there is variation between the change in fluorescence when VSV is added to the sample containing aptamer and cells that have been pre-incubated for 30 minutes at 37˚ C. VSV was added one hour before flow analysis was to take place. Overall there is a general increase in fluorescence which is an unexpected result. This experiment was performed twice to ensure that there was in fact an increase in fluorescence when the virus was added meaning the virus was not displacing the aptamers or the aptamers had a stronger affinity for the binding site than the virus.

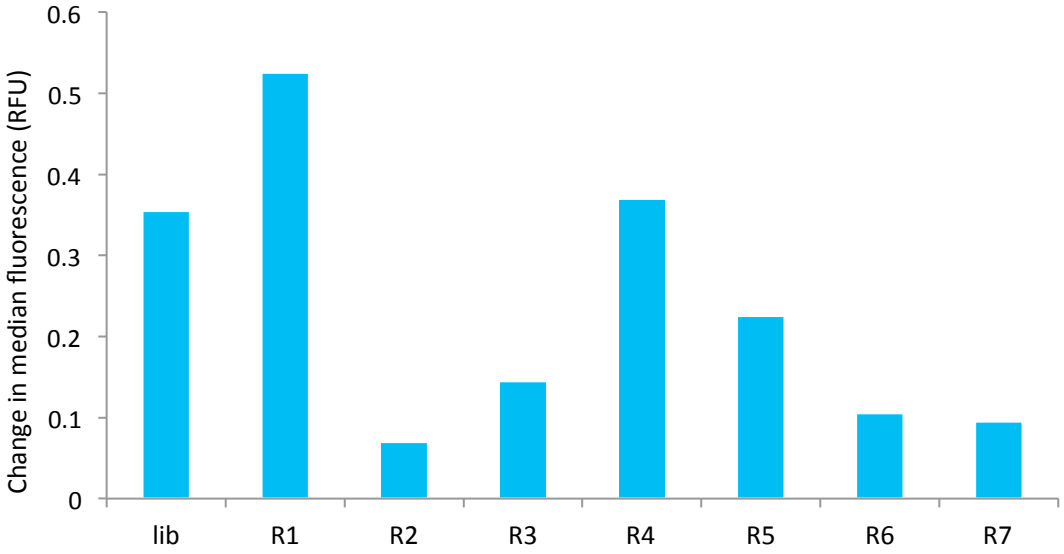

Figure 7. Change in median fluorescence intensity between cell samples incubated with aptamer and VSV from samples containing just cells and aptamer. Fluorescence was detected by flow cytometry analysis after a 1 hour incubation with VSV.

In an attempt to fix this problem, VSV was incubated with the aptamer-cell complexes for a shorter time period shorter thanthat of selection (around 30 minutes before flow analysis). The results from this flow analysis showed overall the same results in which the samples with VSV added had a larger fluorescence intensity compared to samples with just aptamer-cell complexes. The results can be visualized in Figure 8. Note that there are a few exceptions and this could be due to the shorter incubation time of the virus.

Figure 9 confirms that the Vero cells incubated with VSV do produce their own fluorescence compared to the control Vero cells. There is a fluorescence change between the control samples where the Vero cells alone show a significantly decreased fluorescence signal compared to the Vero cells that have been infected with VSV. The fluorescence signal is most likely being produced by the yellow fluorescent protein (YFP) that is encoded in the VSV genome (VSV-YFP used by the Berezovski lab). Other studies have shown that recombinant Vaccinia virus for example, can have a positive correlation of green fluorescent protein (GFP) expression and aptamer signals [9].

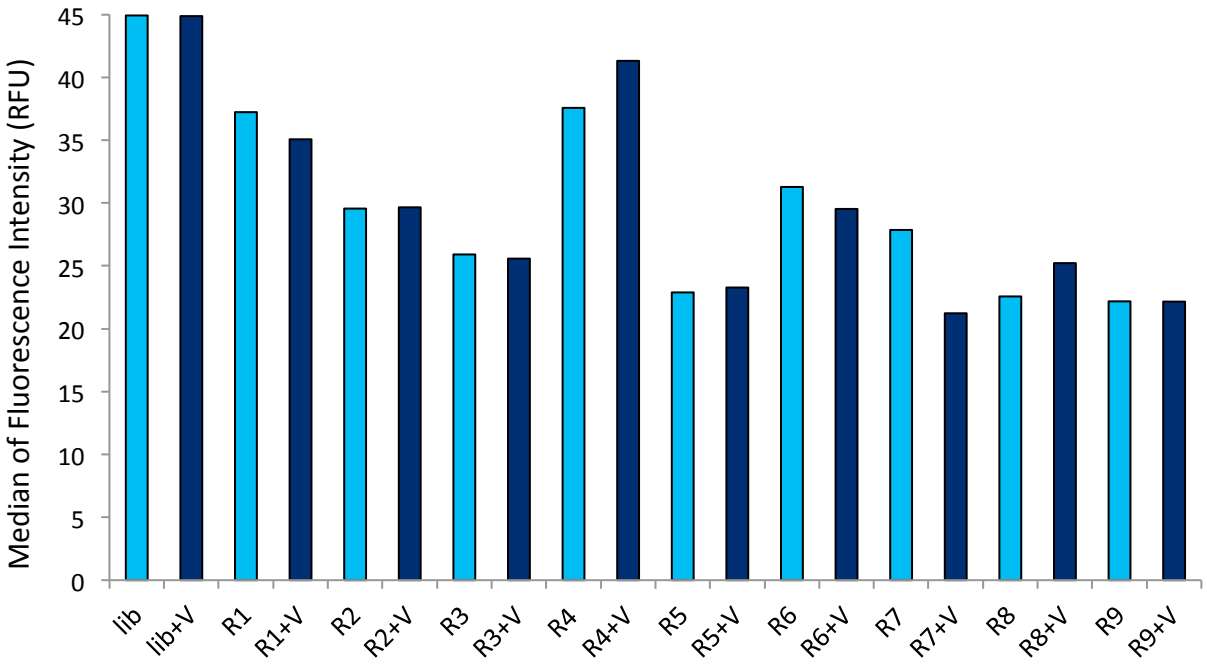

Figure 8. Median fluorescence intensity detected by flow cytometry after washing aptamer and cells with medium and incubating half the sample with VSV for 30 minutes.

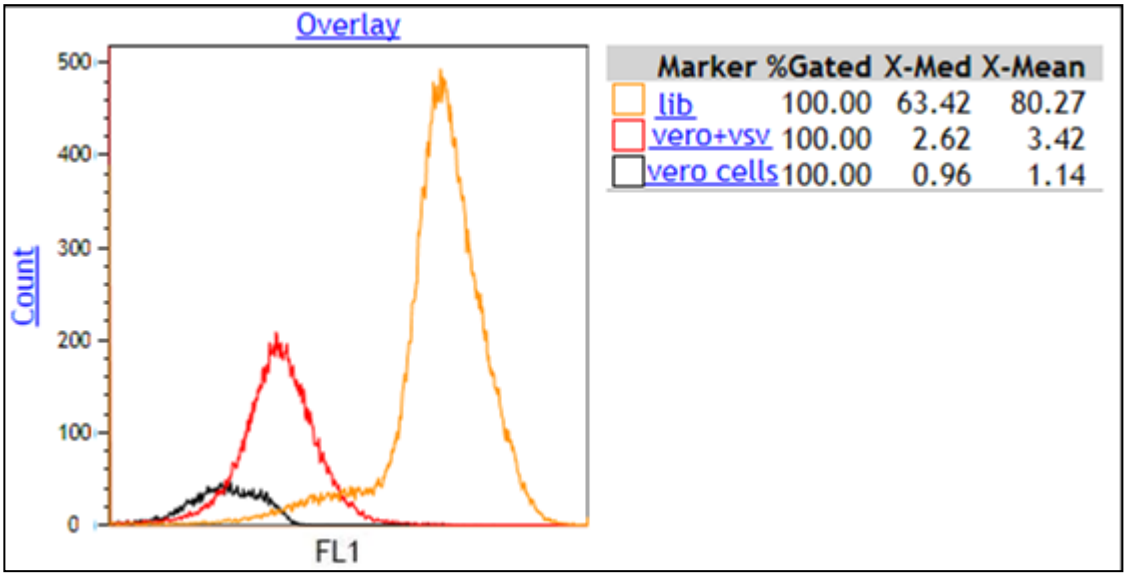

Figure 9. Flow cytometry overlay showing the measurement of fluorescence intensity of Vero cells incubated with aptamer pools 1, 7 and 9 compared to the Vero cell control. The cell count shows the number of cells analyzed.

Further research was done to determine if the emission spectrum of 5-FAM (carboxyfluorescein) which is used to fluorescently label the aptamers during PCR could in fact be overlapping with the YFP emission spectrum from VSV causing an increase in fluorescence signal when VSV is added to the aptamer-cell mixtures. This possibility was analyzed using Life Technologies online Fluorescence Spectraviewer (Invitrogen). From the information obtained the fluorescence spectra of the two fluorescent labels, 5-FAM appears to have an emission peak at 517 nm and YFP has an emission peak at 529 nm. These two wavelengths are close enough in range that there could be detection of YFP in the same fluorescence channel (FL1) that is used to analyze fluorescence from the aptamers labelled with 5-FAM.

To analyze the aptamer pools better, it would be best to label the aptamers with a different colour fluorescent dye such as Texas red (Invitrogen, #T1905). This would prevent the overlap of the emission spectrum of the yellow fluorescent protein (YFP) being expressed by the viral genome after Vero cell infection. Another option would be to use unlabelled virus for flow cytometry analysis. For the sake of this project, it may also be possible to add the virus to the samples containing Vero cells and aptamers just a few seconds prior to flow cytometry analysis so that only the displacement of the aptamers takes place and the fluorescence change can be monitored immediately without the impact of YFP from the virus.

The time of viral protein production after Vero cell infection could also be monitored by performing a separate experiment. Vero cells could be infected with VSV at different time points varying from time 0 up to 24 hours. The high sensitivity of the flow cytometer is most likely picking up fluorescent signals that the virus has produced in the cell that are normally not

detectable until up to 24 hours after infection using other fluorescence viewing techniques like the Alphaview imager.

The anticipated results from this future experiment would be that after addition of VSV to the Vero cells that have been incubated with aptamer, there would be a decreased amount of fluorescence detected. This would indicate that the virus has displaced the aptamers from their binding location on the cells and that these aptamers are in fact being selected to biomarkers on the surface of Vero cells. This biomarker would be indicative of the binding site for VSV in interferon deficient cells. This binding site could be further characterized in future studies using mass spectrometry analysis[1]. The binding affinity of the different aptamer pools could also be assessed using flow cytometry analysis.

Eventually the aptamer pools with the highest affinity for the vero cells could be inserted into plasmids for cloning in bacteria and these sequences could then be isolated for sequencing[9].

Conclusions

Based on the experiments performed, we can conclude that cell-SELEX is an effective way to select aptamers for target cells. These aptamers may have indeed discovered a valid biomarker for interferon deficient cells. Since these experiments were performed at 37˚ C, these aptamers also show stability and potential for use in organisms with this body temperature. Aptamers are an excellent alternative to antibodies because of their high stability under varying conditions, their reproducible properties as well as the capacity for optimization and their cost effectiveness.

For the application of these aptamers in cancer detection, we would want the aptamers with the highest affinity for the target to get the most accurate results for cancer diagnosis. If these aptamers positively detected a cancerous cell, the vesicular stomatitis virus could potentially be used as a treatment strategy. Compared to other cancer treatments such as chemotherapy and radiation, the virus shows minimal side effects and could prolong the health of the patient. While these aptamers have a lot more analyses to undergo, they show extraordinary promise as cancer research and treatment tools.

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