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THE RESEARCH CLUB

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Welcome to the Research Club!

The Research Club is created to facilitate student research in a myriad of topics. Every Friday, we will either be listening to a student researcher, doing experiments, learning lab techniques, preparing for competitions, or working on internship applications. It is one of the few clubs in Stuyvesant dedicated to developing student's interest in research and preparing student's for greater opportunities. One of its main goals is to revive Sigma, its magazine, in order for more members of the Stuyvesant community to have access to scientific material written by their peers. By encouraging its members to write articles, the club aims to hone the writing, presentational, and communication skills of future scientists.

Additionally, the club will expand and strive to encompass multiple fields of science research: chemistry, physics, earth science, computer science, etc. The club hopes to motivate its members to actively engage in scientific discussions and earn valuable experience by receiving feedback to improve their scientific writing.

This edition features many of this year's graduating seniors' research reports, which were submitted to Regeneron and various other prestigious competitions.

The reports have been annotated and shortened by members of the Research Club. We hope this can aid your understanding of the report.

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Measuring the Effectiveness of Treatments for Amyotrophic Lateral Sclerosis

Sophia Xia

Annotated by Cindy Zheng and David Voynov

Motor neurons are nerve cells that carry impulses from the brain and the spinal cord to the muslce or gland.

Abstract

Measuring treatment efficacy for neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS) is difficult due to the subtle changes in **motor neurons**. Small sample sizes accompanied by a lack of research done on this topic advanced this issue. Furthermore, data accumulated from experiments testing the efficacy of stem cell transplantation suggest that the treatment temporarily hinders the progression of the disease.

Adipose tissue are loose connective tissues that store energy in the form of fat. It cushions and insulates the body. The ones we are talking about, Adipose tissue-derived regenerative cells, are involved in wound repair activity

Introduction

Amyotrophic Lateral Sclerosis is a terminal neurodegenerative disease that impairs the motor nerves. Scientists have been studying stem cell transplantation as a possible method of treatment. Embryonic cells are effective due to their higher rates of differentiation into neural cells, but they have been shown to lead to the development of tumors. Fetal cells are a good alternative, but due to an ethical dilemma, they are difficult to obtain. Adipose tissue-derived regenerative cells obtained from fat became a good alternative to other cells as they are rather efficient in differentiation (although less than embryonic cells) and aren't very invasive. While stem cell transplantation has been proven safe with a lot of research, the efficiency of the treatment hasn't been quite as extensively tested.

Lumbar laminectomy: Lumbar laminectomy is a surgery that creates space by removing the lamina(part of the vertebra that covers the spinal canal).

TNFalpha: Tumor necrosis factor alpha is a cytokine that promotes inflammation. It binds to its receptor, causing a cascade that activates NFkBeta (influences cell death).

INF-y: Interferon-gamma is a macromolecular complex consisting of soluble cytokine.

IL-1beta: Interleukin 1 beta is a gene that codes for a cytokine that is produced by macrophages. It acts as a mediator of the inflammatory response and is involved with cellular activities like apoptosis.

IL-6: Interleukin 6 is a cytokine produced by leukocytes that functions in inflammation (capable of inducing fever) and the maturation of B cells.

bFGF: Basic fibroblast growth factor is a angiogenic factor and induces miosis in endothelial cells.

MMP-9: Matrix metallopeptidase 9 is a class of enzymes involved in the degradation of the extracellular matrix.

Materials and Methods

TRANSPLANTATION OF ADRC CELLS

This procedure involves the patients undergoing lumbar laminectomy for the purpose of gaining access to the cerebrospinal fluid. Then the adipose tissue-derived regenerative cells obtained through liposuction can be transplanted through an injection into the cerebrospinal fluid. While transplanting the cells did not have any adverse effect on the patients, the results show that the procedure wasn't very effective at improving the patients' conditions. However, the transplantation has the potential to slow down the progression of the disease despite its limitations. There was a decrease in the proinflammatory agents TNFalpha, INF-y, and IL-1beta while there was an increase in anti-inflammatory agents IL-6, bFGF, and MMP-9.

The therapeutic transplantation of stem cells shows to be promising as it is a safe procedure that may slow down the progression of ALS. Early treatment combined with regular transplantations could significantly reduce the disease's rate of progression and extend the patients' lives. The patients were tested in 12-week intervals starting from 24 weeks prior to . treatment until 36 weeks after treatment

Each of them went through muscle strength, functionality, spasticity, fatigue and depression assessments for a more comprehensive and accurate view of the patients' conditions. All the tests mentioned previously were performed every 12 weeks except for depression which was tested in 24-week intervals. While the plethora of tests makes the results more accurate to some degree, the small sample size of only 4 patients hinders this study greatly. Patients should have been sourced from more hospitals/centers/ In addition, the tests should've been conducted more often in order to get a better view of the treatment's effect. This reigns especially true for the first few months after the treatment was administered when it should be most effective.

Spasticity: Spasticity is a condition in which certain muscles continuously contract, causing stiffness and can interfere with normal movements.

TRANSPLANTATION OF MESENCHYMAL STROMAL CELLS

This procedure involves the intrathecal application of BM-MSCs obtained from bone marrow. It was chosen over intravenous application because it has greater efficacy when it comes to delivering the cells to the central nervous system tissue. It successfully slowed down the progression of the disease with significant stabilization of the disease observed in the majority of the patients at the three-month mark after the patients underwent surgery. However, after the third month, there was a regression in the patients' conditions. None of the patients who took part in the study suffered major side effects caused by the treatment. There were a total of 24 patients who took part in the study. Patients were chosen based on whether or not their diagnosis was definite, if their life expectancy exceeded two years, and if they were between the ages 18-65. In addition, patients who had medical conditions that would make the experiment dangerous for them to participate in were excluded to protect their safety. They were evaluated using the ALSFRS, FVC and muscle weakness (WS) scales. ALSFRS is more subjective compared to FVC and WS because it may be affected by the patients' mental health conditions. They were tested 6 months before treatment until 18 months after treatment. However, only ALSFRS was tested at regular intervals. Testing at regular intervals will provide more data for a more accurate view of the progression of the disease. In addition, they should have asked the patients' about their mental state as that has the potential to affect and skew the results.

Mesenchymal Stromal Cells: Mesenchymal Stromal Cells are spindle-shaped cells isolated from bone marrow, fat, and other tissues. These cells can differentiate into a variety of cells, and possess broad immunoregulatory properties.

This change resulted in a proline to leucine amino acid change in the gene's product that seems to have altered the binding pocket of Protein Y and thus perhaps prevented the binding of Compound 1A.

Conclusion

One of the mutants, B4, showed a clear difference in morphology compared to the other mutants and wild type when grown on two different 7H11 agar plates (Figures 1A and 1B). The colony looked flat; this stood in contrast to the other colonies, which looked ruffled (a characteristic stemming from the fact that Msm cells often grow on top of each other). This difference could signal slower growth and/or a cell wall defect. In liquid media, B4 did not show a significant difference in growth (Figure 3),

The mutant genotypes were analyzed through DNa extraction, PCR, and sequencing. One mutant, D4, was found to carry a mutation in Gene YMsm. The mutation was a single nucleotide substitution that resulted in a proline to leucine amino acid change (P76L) in the gene product (Figure 6). (Figure 8) shows differences in protein structures. In addition, mutant D4 showed complete resistance up to 100 μM of Compound 1A (Figure 4), while the other mutants were eventually inhibited.

In this study, 56 Msm mutants resistant to Compounds 1A and 1B were isolated. Based on colony morphology on 7H11 agar, eight mutants were chosen for further experiments. Out of these eight mutants, one of them (B4) showed a striking difference in colony morphology on 7H11 agar. Finally, the DNA of the eight mutants was extracted for use in PCR. PCR amplification and sequencing for Gene YMsm in Mutant D4 showed a nucleotide substitution.

Accessory proteins: Encoded by the retroviral genome. Sometimes inhibit replication in cultured cell lines, but assist in viral replication.

Cloning and Expression of HIV-1 Vpu

Apurva Singh

Annotated by Thibaud Roy and Karen Chen

Abstract

To establish a productive infection, HIV-1 must counteract host factors that inhibit viral replication. To do this, HIV-1 encodes accessory proteins that modulates the host cellular environment to optimize viral replication and avoid host defenses. Vpu, an accessory protein, is an important component of HIV's pathogenicity. Vpu has several important functions that increase HIV-1 production and may enhance pathogenicity *in vivo*. Vpu, accomplishes this in part by antagonizing the activity of the host proteins involved in defense8. In this study, we focused on understanding how the amino acid residues in Vpu affect its expression. To accomplish this, we introduced mutations in the C-terminus of pNL4.3 Vpu by PCR. The mutated fragments were cloned into expression vectors that coexpressed the green fluorescent protein (GFP). Seven Vpu mutants were then generated and their expression was verified by transfecting human embryonic kidney cells and subsequently performing western blotting.

Pathogenecity: the ability of an organism to cause disease

PCR: Polymerase chain reaction. A reaction that uses the ability of DNA polymerase to synthesize new strand of DNA complementary to a specific template strand. At the end of the PCR reaction, the specific sequence will be accumulated in billions of copies.

Introduction

Although Human Immunodeficiency Virus (HIV) gained momentum in the latter half of the 20th century, it is still a health issue that affects millions of lives globally. Nearly 37 million people were living with HIV at the close of 2017 with 1.8 million of those people getting the virus just that year. In that same year, approximately 1 million people died from illnesses associated with HIV. HIV affects populations all over the world, but some are more affected than others: the continent of Africa accounts for almost 70% of the people infected with HIV5.

CD4 Cells: a class of T cells that secrete small molecules known as cytokines that regulate the activity of other immune cells

HIV is a virus that weakens the immune system by destroying CD4 cells, crippling the body's immune system and leaving it unable to fight the pathogens. Over time (anywhere from 2 to 15 years) a person's count of functional CD4 T cells may become so low that they are unable to fend off even the simplest infections such as the cold and flu. This is the most severe stage of HIV and is termed AIDS or Acquired Immunodeficiency Syndrome. When patients reach this stage of HIV infection, they often also suffer from other diseases/illnesses such as tuberculosis and meningitis. Fortunately, complete elimination of HIV can be achieved through the development of an effective HIV vaccine. One potential therapeutic cure involves the HIV accessory protein Vpu. It has been shown that Vpu also induces the degradation of CD410 and counteracts the host antiviral factor tetherin2. This study sought to understand the significance of the Vpu sequence heterogeneity and its influence on Vpu functions. The results of this study may help us identify new novel targets or pathways that can be blocked to prevent the establishment of HIV-1 infection and/or persistence.

For the last 20 years, studies have been underway to develop HIV vaccines that will prevent the virus from spreading. Although a vaccine may not provide relief for those already suffering with HIV, it will ensure that the virus does not transmit and affect other people. This could lead to the global eradication of HIV which would save millions of people from ever having to experience this devastating disease. The HIV vaccine efficacy trials conducted so far have not been successful with the exception of the RV144 clinical trial. It was the first trial that showed a vaccine efficacy of 31.2% in a period of 42 months. Further studies using this vaccine however, did not yield the same results. Nonetheless, the trial established the possibility of developing a vaccine in the future.

Antiretroviral Therapy (ART) is currently the most effective treatment on the market. However, it is not a cure as it has to be taken consistently throughout one's lifetime to keep the symptoms of HIV at bay. If one misses a single dose of the drugs associated with ART, it could be costly. If this is consistently a problem, they may develop a resistance to the drug. This could perhaps lead to them resorting to a less effective drug. In addition, there are numerous side effects that may accompany the use of these drugs. Clearly, while the treatments available are certainly effective in helping a person live with HIV, they have their drawbacks. This is why there are many experiments going on to discover a cure for HIV. One potential therapeutic cure is the HIV accessory protein Vpu.

Accessory protein: a protein that works with another protein (e.g. helps it fold, anchors it to the membrane, stabilize the primary protein)

HIV-1, a complex retrovirus, encodes accessory proteins (Nef, Vif, Vpu, Vpr, and/or Vpx) in addition to the structural (Gag, Pol, Env) and regulatory (Tat, Rev) proteins. Vpu, which is an 81 aa (16 kDa) type I integral membrane phosphoprotein is an important component of HIV's pathogenicity and is expressed from a bicistronic mRNA together with envelope protein (Env) during the late stage of the viral life cycle. Vpu has several important functions that increase HIV-1 production and may enhance pathogenicity in vivo. It has been shown that Vpu also induces the degradation of CD410 and counteracts the host antiviral factor tetherin2. This study sought to understand the significance of the Vpu sequence heterogeneity and its influence on Vpu functions. The results of this study may help us identify new novel targets or pathways that can be blocked to prevent the establishment of HIV-1 infection and/or persistence.

Integral membrane phosphprotein: a type of protein that is permenantly attached to the cell membrane

Bicistronic mRNA: an mRNA molecule that encodes only two proteins

The research that has been done on the Vpu protein so far has focused on its potential to alter the natural ionic currents that move across the plasma membranes of our cells. This function of Vpu has led scientists to look for inhibitors of Vpu which could stop the virus's transmission throughout the body. The first inhibitor that was discovered was 5-amiloride (HMA). With further testing, it was discovered that it was not as effective as previously thought because of its low selectivity index. A second ion channel activity inhibitor, BIT225 exhibited a high selectivity index and entered phase two clinical trials a few years prior4. By inflicting mutations in the Vpu region of the HIV genome, we hope to discover more about the way in which the Vpu protein allows for viral progression and find additional Vpu inhibitors that could counteract the function of the Vpu accessory protein

Sensitivity index: a statistical value used to evaluate whether or not a signal can be easily detected.

Materials and Methods

Plasmid Constructs

Several primer pairs were designed to create point mutations in the Vpu protein of HIV-1. These primer pairs are shown in Table 1. These primer pairs were synthesized commercially. Schematics of vector plasmid DK-11-14 (pNL4.3) as shown in Figure 1, was used to clone the Vpu PCR fragment with mutation. To amplify the Vpu fragment with point mutation, Polymerase Chain Reaction (PCR) was performed using reaction mixtures containing each primer pair mixed with the GoTaq green master mix. PCR products were purified from the gel using an invitrogen gel extraction kit. The plasmid DNA and Vpu PCR products were digested with restriction enzymes XbaI and MluI. The digested products were then purified again and ligated using the DNA ligase provided by (Invitrogen). Ligated mixtures were transformed in bacterial cells (Top10, Invitrogen) and plated on LB agar containing ampicillin. Several ampicillin resistant clones were tested by PCR. Clones containing the correct Vpu fragment were selected using nucleotide sequencing. Based on the sequencing result, correct clones were identified for transfection and western blot analysis.

Cell line, Protein Expression and Western Blot Analysis

HEK293T cells were obtained from the American Type Culture Collection (ATCC) and used for transfection. This cell line was propagated in DMEM media with penicillin streptomycin, fetal bovine serum, and L-glutamine. The selected plasmids were tested for the expression of the 4 recombinant proteins by transfecting 293T cells with the different mutant plasmids using jetPEI transfection reagent. 48 hrs post-transfection, 293T cells were washed with PBS twice and lysed in 1% empigen. The cell lysates were clarified by centrifugation at 15000 rpm for 10 minutes at 4oC. The clarified supernatant was collected and stored at -80oC until use. The expression of the mutated Vpu was confirmed by Western blot analysis. The clarified cell lysates were fractionated on 10% SDS page gel and transferred to the nitrocellulose membrane. The membrane was blocked with 5% nonfat dry milk in PBS overnight. The next day, after washing with PBS Tween-20, the membrane was incubated with HIV-1 Vpu 6780 and AU1 antibody for 45 minutes. The membrane was then incubated for 30 minutes at room temperature with anti-mouse-HRP and anti-rabbit-HRP secondary antibodies to detect the binding of HIV-1 Vpu 6780 and AU1 antibody respectively. The membrane was washed, and bands were detected using chemiluminescent substrate and visualized using Bio-Rad Imager.

The effects of LDH-A inhibition on the metabolism of prostate cancer

Kelly Kang

Annotated by Justin Lam and Kristoff Misquitta

Abstract

Lactate dehydrogenase (LDH) is an enzyme found at the end of the glycolytic pathway in nearly all living cells. While playing an important role normally in healthy cells, it also plays a significant role in the proliferation of most cancer cells. This study attempted to better understand the effects of LDH on cancer cell metabolism. In the absence of LDH, measured changes in pH, lactate, glucose, and growth were used to compare the metabolism of bulk cells to knockdown cells. It was confirmed that LDH-A knockdown shows a trend towards inhibition of proliferation of prostate cancer cells and tumor growth. This implies that LDH-A knockdown may be an effective strategy to reduce tumor proliferation.

Introduction

Metabolism of cancer cells relies heavily on glycolysis. Thus, it is marked by significantly high lactate dehydrogenase activity. Lactate dehydrogenase (LDH) is an enzyme in the glycolysis pathway that catalyzes the interconversion of pyruvate to lactate. It has been well documented that the dysregulation of lactate synthesis metabolism and lactate signaling contribute considerably to carcinogenesis. The relationship between glucose utilization and lactate exchange then allows for cancer cells to grow and proliferate at their abnormally accelerated rates. The role of the LDH enzyme is to catalyze the conversion of pyruvate to lactate (see Figure 1).

Glycolytic pathway refers to glycolysis, or the extraction of energy from the breakdown of glucose.

Prostate cancer is the cancer of the prostate gland, which produces seminal fluid in men.

LDH-A knockdown means that the gene responsible for the synthesis of LDH is removed from a cell sample, preventing the cell from producing the enzyme LDH.

Pyruvate is the 3-carbon end product of glycolysis. In the presence of oxygen, it progresses into the mitochondrial matrix to be oxidized.

Carcinogenesis is the formation of cancer cells.

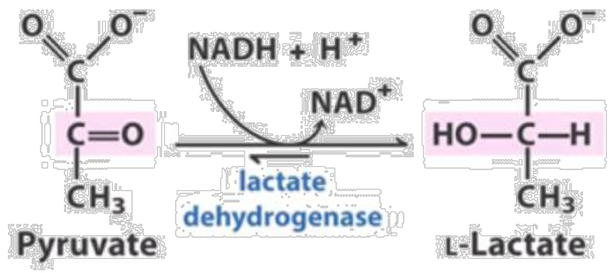


Figure 1

Isomers are chemical compounds that have the same number and types of atoms, but the atoms are arranged differently.

Isoenzymes are enzymes with different structures, but with identical functions. The enzyme is composed of four monomers, so it is called a tetramer.

Murine refers to mice. These "cell lines" are specially designed samples of prostate cancer cells from mice.

In vitro means that cell lines are observed inside a test tube or plate. ***In vivo*** means the cell lines were observed inside an organism.

The procedure describes the conditions (pH and glucose concentration) that the cell lines were grown under.

Commonly abbreviated RNAi, **RNA interference** is carried out by the binding of small interfering RNA (siRNA) to mRNA, marking it to be cleaved.

Seeding is the transfer of cells onto a new surface or container.

Lysis solution dissolves the cell membrane, creating a "cell lysate" of DNA and proteins.

When pyruvate is converted to lactate, the process of lactic acid fermentation can then occur. It is a metabolic process by which glucose and other six-carbon sugars are converted into cellular energy. Two isomers, *LDH-A* and *LDH-B* , form two 5 active isoenzymes (tetramers). Inhibiting LDH-A expression through knockdown and studying the effect in tumor metabolism and the tumor microenvironment has therapeutic benefit, such as improving CAR T cell treatment.

Past experiments have studied the effect of LDH-A knockdown in a murine breast cancer. The focus of my current research is to then demonstrate a more accurate depiction of the metabolic activity in murine MycCaP prostate cancer cell lines, transduced with a vector coding for the human prostate specific membrane antigen (PSMA+). It is certain that PSMA has an effect on prostate cancer proliferation, as prostate cancer cells have high levels of prostate-specific membrane antigen (PSMA), although its biological role is still uncertain. We first developed *in vitro* and then *in vivo* growth profiles of the MycCaP cell lines to study the effect of LDH-A knockdown on cell proliferation and tumor growth. These studies are followed by metabolic assays to measure glucose utilization, lactate production and pH changes in the MycCaP cell lines.

Materials and Methods

Cell Culture

hpSMA+ MycCaP sh RNA knockdown clone (A2-2 KD) and hPSMA+ MycCaP scrambled control (A5NC Bulk) were cultured in DMEM + 10% FCS P + S media (5 mM and 25 mM glucose).

The culture media was fixed to pH 7.4 and monitored for change in pH on the first and third day. The pH meter was kept in the culture media for 30 seconds and the pH measurement on the pH meter was then recorded.

The culture media was also simultaneously monitored for glucose concentration with an Accutrend glucometer. To do so, 15 µl of the culture media was pipetted onto a glucose strip, was was then read by the Accutrend glucometer, returning an exact value of glucose concentration.

LDH Knockdown

The LDH gene is knocked down through RNA interference, which is a means of silencing genes by way of mRNA degradation.

LDH Assay

LDH enzyme assay was conducted with Roche Scientific LDH Assay Kit by seeding 104 cells and assessing enzymatic activity.

We split cells from the plate and counted the number of cells present in 1 mL. Meanwhile, we calculated the number of cells needed and the serial dilution scheme. We took the highest number of cells needed for the assay and transferred to the tube. We made up the volume to 1 mL with the same media. s we have 3 replicates of each cells and we were using only 100 µl per cell, we needed 300 µl of cells for plating. The rest of the cells were used to make serial dilution. After all the cells were counted and serial dilutions were made, we plated 100 µl of cells in a 96 well plate, with the location of cells in each well noted. The plate was incubated for 2 hours to let cells sit down. 5 µl of lysis solution was added to each well with multi-channel pipetter. Again, there was an incubation

period of of 15 minutes at 37 degrees. Meanwhile, we prepared the reaction mixture. We counted the number of tests (each well equals to one test) and made up the mixture according to that. The ratio of catalysis to dye was 1:45. Then, we added 100 µl of the freshly prepared reaction mixture to each well and incubated for 10 minutes at room temperature. The dishes were covered with aluminum foil to prevent light exposure. Finally, 50 µl of stop solution was added to each well. The ELISA reader was used to measure the absorbance of the samples at 492 nm.

ELISA stands for Enzyme-Linked Immunosorbent Assay. It involves the binding of a series of highly specific antibodies to an antigens. The final antibody is bound to an enzyme that converts a substrate into a measurable colored product.

In Vitro Growth Assay

In vitro cell proliferation assay was done by seeding 10^6 cells and monitoring growth over 3 days.

In Vivo Growth Assay

Tumor growth assay was done by subcutaneously injecting NOD SCID mice with 2x10^6 cells in the right flank and measuring growth by caliper. The volume of the tumor was calculated using the dimensions of the tumor, as measured by the caliper.

Lactate Assay

Cells and culture media were deproteinized and lactate was measured with an Eton Bioscience L-lactate Colorimetric Assay Kit.

Reagents Preparation

1. Lactate Standards: The vials contains 1000 µl of 3mM L-Lactate Standard. The standard was equilibrated to room temperature before use. 1mL of the standard makes 3 standard curves when assayed in duplicate.
2. L-Lactate Assay Solution: The solution contains enzymes that are light sensitive. The solution is thawed on ice before use.

Sample Deproteinization

1. We took 400 µl of media PBS serum sample and added 100 µl ice cold PCA.
2. We placed samples on ice for 5 minutes.
3. Centrifuged at 13000g for 2 minutes.
4. Transferred 380 µl of the supernatant to a fresh tube.
5. Added 20 µl of ice cold neutralization solution to the samples and mixed

Adding Samples:

1. Added 50 µl of samples to each well. Assay samples in duplicate.

Standard Curve Preparation

1. A standard curve must be run in each assay
2. Added 50 µl, 40 µl, 30 µl, 20 µl, 10 µl, 5 µl, 1 µl, and 0 µl of L-Lactate Standard to each well for a total of 8 standards. Then adjusted volume to 50 µl well with dH2O.

Perform the assay

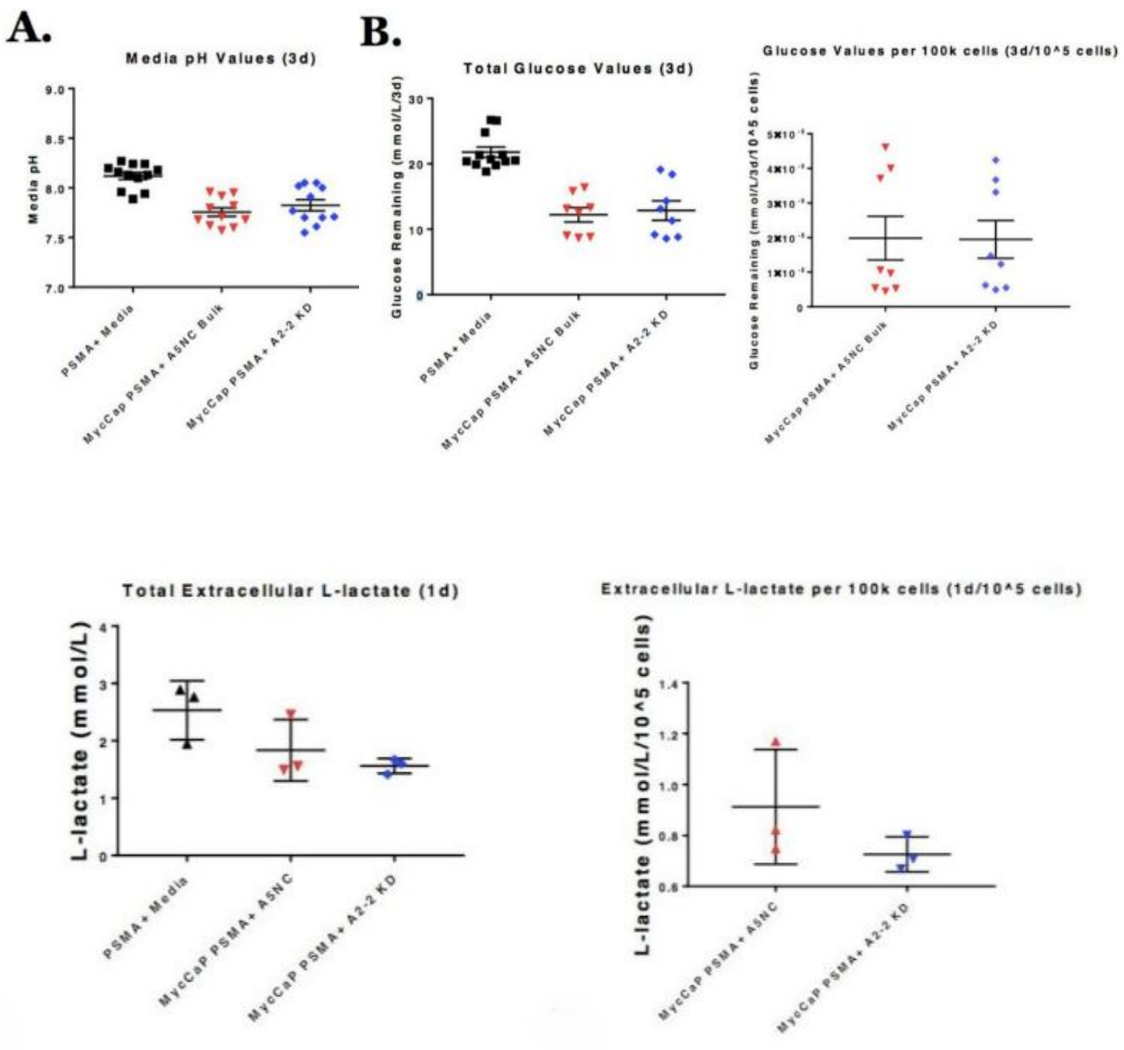
1. Added 50 µl of L-Lactate assay solution to each well containing L-Lactate standards and test samples.
2. Incubated for 30 mins at 37 degrees Celsius incubator.
3. Stopped the reaction by adding 50 µl of 0.5M acetic acid per well followed by brief gentle agitation.
4. Measured the absorbance at 490nM using a microplate reader.

Calculations

1. Averaged the OD490 nm values of replicate wells of each L-Lactate standard, test samples, and blank. In order to get the corrected absorbance, we subtracted the average OD490 nm value of the blank from the average OD490 nm values from all standards and samples.
2. Made a standard curve by plotting OD490 nm values from each L-Lactate standard as a function of L-Lactate concentration.
3. Calculated the value of L-Lactate in samples using the equation obtained from the linear regression of the standard curve.

   L-Lactate (µM) = [(Corrected absorbance) - (y-intercept)]/slope

Results



Conclusion

LDH-A knockdown shows a trend towards inhibition of proliferation of MycCap prostate cancer cells and tumor growth. LDH-A knockdown also shows a trend towards reduction of extracellular lactate production, extracellular acidification, and glucose utilization. LDH-A knockdown may be an effective strategy to reduce cancer cell proliferation *in-vitro* and potentially *in-vivo*.

We are able to identify quantitatively the metabolism of the cells due to the fact that the pH of MycCap PSMA+ A5NC Bulk and the MycCap PSMA+ A2-2 KD are lower than the control PSMA+ Media, which did not have cells in it. The release of CO2 by the cells is what decreases the pH.

Therefore, it follows that the faster the metabolic rate of the cells, the lower the pH would become. The data here makes sense as we see the metabolism of MycCap PSMA+ A5NC Bulk cells is faster than that of the MycCap PSMA+ A2-2 KD cells, which had the LDH enzyme knocked down, affecting its conversion between pyruvate and lactate.

Additionally, we are able to identify quantitatively the metabolism of the cells due to the fact that the glucose of MycCap PSMA+ A5NC Bulk and the MycCap PSMA+ A2-2 KD are lower than the control PSMA+ Media, which did not have cells in it. In order to sustain themselves, the cells use up glucose in the media, therefore decreasing it. However, the number of cells in the media affects the amount of glucose that is uptaken. As a result, the graph on the right of Figure B is adjusted to the number of cells. After adjustment, we are able to identify quantitatively that after 3 days, the glucose values between the two cell lines is not very different.

Differences in cell growth rate (doubling time, DT) were obtained for KD and NC cells. These studies demonstrated that LDH-A KD was the dominant factor in reducing cellular growth: Doubling times were 17.2±0.6 hours (KD) and 14.7±0.5 hours (NC), p = 0.01. LDH-A depletion (KD) resulted in lower, extracellular acidification (pH: NC: 7.76 vs. KD: 7.82; p = 0.01). However, glucose utilization and extracellular lactate production measured *in vitro* showed only marginal differences between KD and NC cells. Nevertheless, the marginal differences could be attributed to mechanisms which will be evaluated in future studies.

Expanding, characterizing and repurposing the invertebrate virosphere

by Benjamin tenOever, Ph.D., Ethan Lin

Annotated by Tyseen Murad

Abstract

Modern medicine demands the capacity to deliver genetic or biological cargo to specific cell types. Past efforts to achieve this goal have relied on the retooling and re-engineering of a small subset of vertebrate viruses with limited success. Remaining challenges with regards to *in vivo* delivery include finding novel viral vectors that can achieve different target specificities in addition to those that are more amenable to synthesize *de novo*. In an attempt to address these remaining limitations, we propose to collect and sample diverse invertebrate species to isolate and identify RNA viruses associated with them. As the invertebrate virosphere remains largely unknown, we hypothesize that we will identify novel viruses, whose components can be characterized and repurposed to build a new suite of viral-based tools. To this end, we propose to isolate and sequence RNA from a diverse library of invertebrates by next-generation sequencing and subsequently perform *de novo* genome assembly on the reads obtained. Captured reads will then be analyzed for signatures of RNA dependent RNA polymerases (RdRps) – a necessary component of all RNA viruses. Putative novel virus genome assemblies will be named, characterized, and independently confirmed by quantitative PCR. Small RNA viruses or their RdRps (less than 5kB) will then be synthesized and artificially launched in mammalian cells to ascertain whether they can be selected via guided evolution to function and deliver a desired genetic or biological cargo.

Viral vectors are tools commonly used by molecular biologists to deliver genetic material into cells.

Polymerase chain reaction (PCR) is a method widely used in molecular biology to make many copies of a specific DNA segment. Using PCR, a single copy (or more) of a DNA sequence is exponentially amplified to generate thousands to millions of more copies of that particular DNA segment.

Lentivirus is a genus of retroviruses that cause chronic and deadly diseases characterized by long incubation periods, in the human and other mammalian species.

Introduction

In the advent of recombinant DNA, technology science has witnessed the development of tools to generate antibodies from a plasmid, silence messenger RNA, deliver genes, and even edit DNA with single base pair resolution. However, to capitalize on these discoveries, the scientific community needs the ability to deliver the necessary cargo to the cells of interest. Thus far, the issue of delivering genetic material to cells has come in the form of either repurposed viral vectors or the direct delivery of genetic material (Thomas *et al. 2003*).

With regards to viral vectors, these have largely focused on the use of lentiviruses such as HIV, adenovirus, or the adeno-associated virus. While each of these viral vectors has demonstrated promise in some context, each has inherent issues preventing their widespread use.

With regards to viral vectors, these have largely focused on the use of lentiviruses such as HIV, adenovirus, or the adeno-associated virus. While each of these viral vectors has demonstrated promise in some context, each has inherent issues preventing their widespread use. For example, early clinical trials with lentiviruses resulted in multiple integration events that culminated in the development of cancer. Conversely, use of adenovirus, while it does not integrate, many in the human population have pre-established immunity to the vector rendering it ineffective. While both seroprevalence and integration are issues that can be addressed with additional testing or methodology, neither vector represents a tool that can be used in a more generalized platform. In place of these two popular vectors, a third expression system has gained popularity called the Adeno-associated virus (AAV). This vector, which in nature impacts canines, can be repurposed for gene delivery and has shown significant promise in clinical trials. While AAV neither integrates nor shows high seroprevalence, its limitations derives from the fact that it tends to deliver to the liver and has a very limited coding capacity.

Adenoviruses are a group of common viruses that infect the lining of your eyes, airways and lungs, intestines, urinary tract, and nervous system.

We still use these vectors despite this limitation but the scientific community is simultaneously looking for other solutions. Over the past few years, many have focused on various lipids or synthetic nanoparticles to deliver recombinant DNA to cells. However, this has proven difficult, largely owing to the inability to breach the barriers required to reach the nucleus. More recently. a promising technology in this area is the direct use of RNA. The use of RNA as a therapeutic is promising in that it can be easily manufactured and neither integrates, nor is it immunogenic. However, while promising, a remaining limitation of RNA is its inherent instability. In this regard, the identification of novel RdRps may also enable the engineering of self-replicating RNAs, thereby overcoming this limitation. In an effort to find a small RdRp that will not show any prevalence in the human population, we propose to sample invertebrates for novel RNA viruses from which we can build.

Specific Aims

1. The initial aim of this project is to gather RNA samples from variegated sources. Multiple samples from a wide range of invertebrate species will provide the necessary heterogeneity from which RNA will be isolated. Subsequent construction of a diverse invertebrate RNA library will allow for the identification and classification of viruses present within each sample (regardless of genome type). The RNA library will then be used to sequence, assemble and identify putative viruses.
2. Upon identification of a previously unknown virus, compatibility with cloning and its evolutionary relationship to other known viruses will be assessed. As previously stated, small RNA viruses and/or viral RdRps that neither integrate nor have a high seroprevalence are ideally suited to work with and advance. Subsequent cloning via synthetic biology and launching in permissive cell lines will serve as the next steps in the progression and development towards a self-replicating RNA.

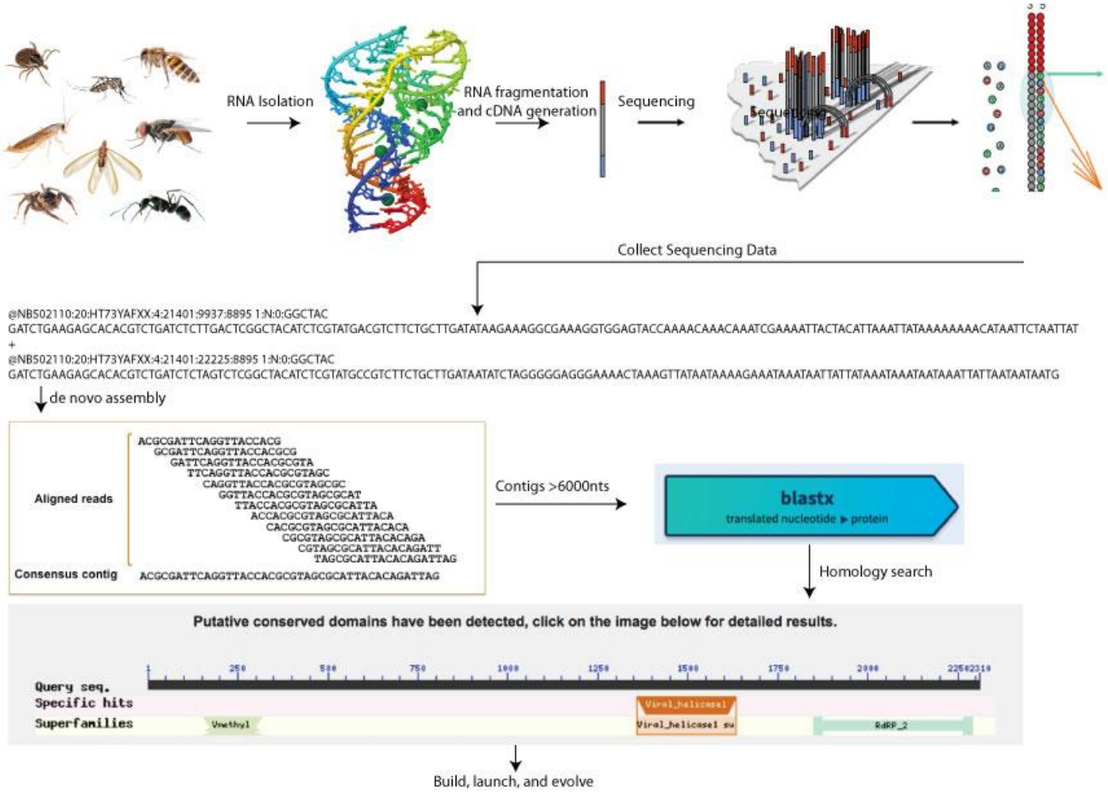


Figure 1. Animation depicting the overall strategy of this proposal. The process begins with the collection of invertebrates. The samples then undergo RNA isolation in order for us to sequence them. In the sequencing step, the RNA is fragmented into many pieces before being amplified many times. The fragments will contain overlapping segments of genetic code, making them amenable to reconstruction via de novo assembly. The subsequent consensus contigs vary in length, but the longer ones (>6000 nucleotides (nts)) will be analyzed via Basic Local Arrangement Search Tool (BLAST) to contrast them with known viruses and determine if they are novel viruses. Putative small viruses and/or RdRps will then be synthesized and introduced into mammalian cells to determine if we can select them to function (this step is denoted as Build, launch, and evolve).

Methods:

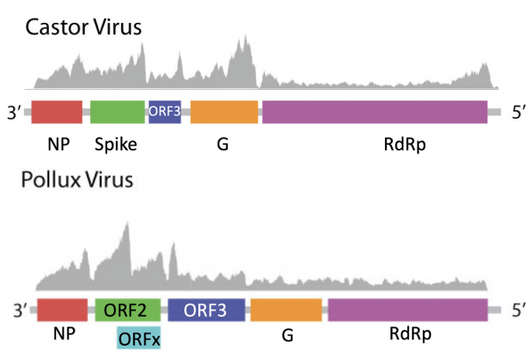
RNA Isolation from Collected Samples: To address the first aim, insects will be collected and stored in RNALater® from predetermined environments in a set area and recorded as a sample ID and suspected species using www.amentsoc.org/insects/what-bug-is-this/. The collected insect will then be pulverized with small quantities of TRIzol reagent, with the exact amounts dependent on total sample size. Subsequent incubation allows for the phenol in TRIzol to break down cellular components while maintaining RNA integrity. Chloroform is added to the solubilized RNA to induce phase separation, which occurs over a 15 minute period of centrifugation. The generated supernatant contains RNA in the colorless upper aqueous phase and can be transferred out of solution via pipetting. The red organic proteinaceous layer and DNA interphase layer are discarded. A quantity of isopropanol, equal to half the added amount of TRIzol Reagent, is mixed into the aqueous solution and allowed to incubate, and the insolubility of RNA in isopropanol will yield a white RNA pellet, albeit impure. Subsequent resuspension in 80% ethanol allows for purification of the RNA due to ethanol's low dielectric constant and propensity of the salt to dissolve in water and force it out from the RNA. The remaining pellet of RNA will then be characterized using a Nanodrop Instrument®. This RNA will then be cataloged and stored at -80.

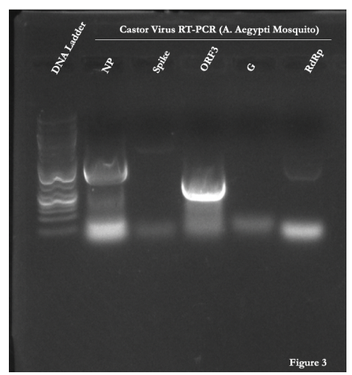
Next Generation Sequencing: High-quality RNA isolated from the previous step (as determined by the Nanodrop) will be fragmented and used to generate an Illumina-compatible library for massively-parallel sequencing (see Figure 1). In brief, each captured RNA fragment will be used to amplify an isolated pool of identical cDNA fragments that can be sequenced alongside each other. Using a high-resolution camera and real-time primer-mediated extension, the NextSeq Illumina instrument can generate 500 million reads from a single run. RNA samples will, therefore, be cloned and processed in this way and sequenced and assembled de novo to identify contiguous RNAs that are greater than 6000 nts in length (as this exceeds the size of most mRNAs whereas viruses are commonly larger than this). "Contigs" will then be translated in all 6 possible frames and putative proteins (larger than 600aa) will be used in a BLASTx search to determine whether there is any homology to known RNA dependent RNA polymerases (RdRps) which are generally larger than 600 residues. Sequenced contigs showing homology to known RdRp will then be characterized to identify additional open reading frames (ORFs). Each putative ORF will be aligned to known viruses and will be fitted into a phylogenetic tree to ascertain which family of viruses it is contained within.

Virus-based rescue systems: Should we identify a novel virus of interest, we will next verify expression within its cognate RNA sample to ensure and verify its sequence. The putative virus will subsequently be PCR amplified and subcloned into a plasmid suitable for in vitro transcription and/or eukaryotic expression. Recombinant RNA or the plasmid will then be introduced into insect or mammalian cells (C6/36 or BHK cells, respectively), and we will use PCR to determine whether evidence of self-amplification can be observed. Should we see some levels of 'replication', we will continue to passage the virus to determine whether we can guide its activity and study its biology. Should this be the case, we also wish to clone the RdRp in isolation to determine whether we can achieve a functional enzyme in mammalian cells that may one day serve to build a self-amplifying RNA.

Results as of June 2019:

The Dioscuri: Castor and Pollux are two viruses that we have recently discovered. They are named that way because of their high homology rate. Both are negative single-stranded RNA viruses that boast a suite of new receptors that could be used for genetic editing. Found below are graphs of the reads indicating there structure and a gel indicating that the virus could be replicated.





Associations Between Brain Connectivity and Symptoms in Co-Occurring Attention-Deficit Hyperactivity Disorder and Reading Disorder

Senjuti Gayen

Annotated by Kristoff Misquitta

Abstract

Attention-Deficit Hyperactivity Disorder (ADHD) and Reading Disorder (RD) are learning disorders that commonly co-occur (Kaplan et al., 2006). Studies that use neuroimaging techniques, such as magnetic resonance imaging (MRI), are ideal for providing information about brain structure and function. Many MRI studies explore alterations in brain connectivity (functional interactions between regions of the brain) in clinical populations, thereby providing biological insight into the disorders. A better understanding of ADHD and RD would allow for the development of preventative treatment strategies and for better medication that specifically target symptoms of ADHD and RD. In this study, twelve children diagnosed with ADHD and RD, as well as twenty-one typically developing (TD) children, were recruited. Participants completed neuropsychological testing and underwent resting-state functional connectivity MRI (rs-fcMRI) scans. The brain scans were analyzed to identify connectivity in the brain associated with symptoms, using various seeds from different brain networks. The findings of the current study support the idea that increased functional connectivity between the cognitive control networks and the default mode network (DMN) is associated with ADHD; as symptoms increased, so did connectivity. In association with reading ability there was connectivity between the DMN and a cognitive control network. The connectivity between ADHD with ADHD and RD.

MRIs use a magnetic field to align protons in the body. Radio waves are then used to force the protons into an energetically unfavorable rotation of 90° or 180°. When the pulse is turned off and the protons re-align with the magnetic field, energy is released that can be used to generate images of internal organs.

Typically developing means developing normally. These children serve as the control in this study.

rs-fcMRI detect changes in blood flow to regions of the brain when no task is being performed.

The Default Mode Network refers to the regions of the brain that remain active at rest.

Materials and Methods

Participants

Twelve children diagnosed with ADHD and RD and 21 typically developing (TD) children were selected from a sample recruited by Columbia University Medical Center/New York State Psychiatric Institute (Davis et al., 2017) The children were all between the ages of 7 and 12 years and were native, monolingual English speakers. Children were excluded from the study if they had taken any psychotropic medication or had any lifetime diagnosis of a neurological or neurodevelopmental disorder other than ADHD or RD. If an RD or ADHD diagnosis was indicated by clinical history and by poor performance (at or below 25th percentile) on neuropsychological measures, children were included in the RD/ADHD group. Two independent licensed psychologists with expertise in the assessment of RD and ADHD reviewed case information to confirm diagnosis and group membership. The Institutional Review Board of the New York State Psychiatric Institute approved this study, including the informed consent and assent procedures for all participants.

Psychotropic drugs include antidepressants and mood stabilizers. They affect brain function, often to treat mental illness.

The **Institutional Review Board (IRB)** approves research involving human subjects using risk-benefit analysis. The approval process considers both ethics and law.

Neuropsychological, reading, and psychosocial measures

A full neuropsychological assessment was administered by a trained research assistant in which children completed the measures described by Davis et al. (2017). The Woodcock Johnson Achievement Tests [WJ-III] (Word Attack) tested for RD and the DuPaul AD/HD rating scale fourth edition (DRS) tested for ADHD.

Imaging data acquisition

Participants in the study were scanned using a General Electric Signa 3-Tesla LX scanner at Columbia University Medical Center. Two T1 structural scans and two 5-minute resting state scans were acquired for each participant (Davis et al., 2017). For resting state image acquisition, participants received instructions to rest quietly and to let their minds wander. An eye-tracking camera allowed the examiner to ensure that participants did not fall asleep during these scans.

Resting State Functional Connectivity Preprocessing and Motion Correction

Analysis was performed in the CONN toolbox v 17.f (Whitfield-Gabrieli and Nieto-Castanon, 2012) for SPM 12 . Preprocessing and motion correction were performed according to the method used by Davis et al. (2017).

Seed-to-voxel connectivity analysis

Blood-oxygen level dependent (BOLD) signal from MRI scans was correlated voxel-by-voxel for each participant over the 10 minutes of resting state data.

Fisher z transformation was applied. Seed-to-voxel connectivity maps were then generated in CONN using four Default Mode, four Fronto-Parietal, seven Salience, and four Dorsal Attention network seeds (Table 1). Seed-to-voxel correlation analyses were used to evaluate any associations between symptom measures (reading ability as defined by performance on the Word.Attack test from the WJ-III or ADHD symptoms as defined by the total current symptoms score on the DuPaul AD/HD rating scale) and connectivity from the above-stated seeds to the rest of the brain. Subsequently, group differences in resting state functional connectivity from the regions that were significantly associated with behavioral measures were assessed via independent two-sample t tests. Findings that reached significance at $p < .05$, corrected using false discovery rate (FDR) correction for multiple comparisons, are presented.

Conclusion

The current study aims to explore the association between symptoms of RD and ADHD and brain connectivity in default mode and cognitive control networks. Given prior work suggesting altered brain connectivity within and between these regions in ADHD populations, I hypothesized that there would be associations between symptom severity and brain connectivity in a sample of children with co-occurring ADHD and RD. Additionally, given the numerous studies that suggest the negative relationship between the DMN and cognitive control networks is altered in ADHD, I hypothesized that our clinical sample would have differential connectivity patterns when compared to typically developing children. The results of the current study suggest that children with co-occurring ADHD and RD have increased connectivity between the DMN and cognitive control networks as clinical symptoms increase. This was seen for looking at both ADHD symptoms and reading ability in relation to brain connectivity.

Particularly for ADHD, connectivity from cognitive control networks to the precuneus cortex was seen in multiple instances: the L LPFC, R LPFC, and L rPFC are regions of interest that displayed connectivity to the precuneus cortex, which is a functional core of the DMN. For RD, the connection was from the R PPC (a part of the FPN) and the cuneal cortex (a part of the DMN). Continuing, there were different brain regions displaying connectivity in association to symptoms and reading ability in the TD group compared to that of the group of children with ADHD and RD; this suggests that different brain mechanisms are underlying attention and reading problems in the kids with clinical disorders. In order to further support such findings, future research should utilize resting state connectivity in a larger sample of children in association with symptoms of ADHD and RD in order to better understand the neural basis of the disorders. As patients with ADHD and RD deal with the effects of the disorders, they also deal with the chances of having a lower occupation status, poor social relationships, and being more likely to develop substance abuse problems. They may also face stigmas and stereotypes working against them and contributing to prejudice and discrimination. Conducting research on the mechanisms and pathways behind ADHD and RD would allow us to be able to develop target solutions and therapies for ADHD and RD. This would aid in the development of preventative treatment strategies for the disorders, and improve their lives above all.

The **precuneus** is a relatively understudied region of the brain, largely due to its location and rare injuries.

Viability of eDNA in a Time Series After Collection

By Neil Sarkar, Shubh Khanna, Seth Cunningham, Michael Tessler

Abstract

Metabarcoding using environmental DNA (eDNA) is making rapid advances when it comes to surveying species in a geographic region. We aimed to find out if time or temperature impacted the quality of eDNA samples in storage. Our hypothesis was that there would be a decrease in concentration as time went on but the decrease would not be drastic. We took samples from the Croton Reservoir and extracted DNA on the same day, as well as 4, 8, and 16 days after sampling. We stored samples at room temperature (and -20 ℃ for a second 16 day sample). This allowed us to correlate DNA concentration/quality over time for these eDNA samples. Our results show that as time went on, the DNA concentration slightly decreased. However, the decrease is not dramatic enough to entail leaving the field to extract the DNA. We also found that freezing does not affect DNA concentration significantly. Furthermore, we used the trnL and 16S genes to do metabarcoding of our samples to look at plant and arthropod species composition differences. Using ordination visualizations, we found little species composition level differences between samples collected at different time points, indicating storage is not an issue. This is significant because it means that researchers do not need to grapple with the issue of collecting samples and immediately leaving the field to extract the DNA. As a result, this study paves the path for future eDNA research and explorations with definitive understandings of how DNA concentration changes while in the laboratory.

Metabarcoding is a molecular biology technique that uses PCR to assess biodiversity from eDNA.

Introduction

The use of environmental DNA (eDNA), genetic material obtained directly from environmental samples (eg. soil, water, etc.), has proven useful for making progress in ecology, evolution, and conservation (Thomsen et al. 2014). eDNA is revolutionizing the field and leading to rapid advances in surveying species in an environment.

Metabarcoding, which is able to look at the DNA of multiple species from a wide variety of organisms in one sample, using eDNA is a growing field of research with the promising potential to distinguish species for general surveys and for ecological studies. We also need to know how best to store and handle eDNA samples. If DNA degrades at an even rate across samples and species, then this loss should have little impact. However, the opposite could be true as the degradation could plausibly be at the point where species composition and abundance loses precision.

Accordingly, our research examined the ability to effectively determine species composition and abundance from eDNA samples collected and stored across a two week time period. Other research has studied similar, but ultimately different, aspects of DNA extraction for this type of work. For instance, regarding the effects of temperature and the amount of DNA obtained, there is a small negative correlation between the temperature at the time of collection and the amount of DNA obtained (Nsubuga, A. M., Robbins, M, et al, 2004). Research on storage temperature, DNA quality, and DNA quantity have also found that preparation of DNA from blood stored at room temperature or incubated at 37 °C for 24 hr resulted in the same amount and quality of DNA as from samples frozen at −70 °C (Debomoy K. Lahiri and Bill Schnabel 1993). Furthermore, another study found that after a month, there is a significant decrease of DNA quality extracted from olive oil with a consequent loss of DNA information, that can affect the reliability of the results (Simona Pafundoa, Matteo Busconib, et al. 2010). Our research is unique in that it is the first to review time of collection for eDNA sequences using NGS metabarcoding.

Previous studies have looked at the preservation of DNA in blood samples and other samples, but they have not examined the factors that affect viability of samples taken from the environment.

Our goals were to understand how long scientists are able to store eDNA samples and identify the ideal storage conditions. We aimed to test the effect of time on the quality and analysis of eDNA samples recorded through metabarcoding. This provides invaluable information to scientists, as it would make it easier to collect more samples in the field without having to worry about immediate DNA extraction in the lab. Thus, time spent obtaining samples will be better able to focus on research and innovations rather than lab work.

Methods

The researchers decided to determine the quality of the eDNA samples by identifying any decrease in the concentration of DNA and how accurate the samples are identifying the source.

In order to determine the quality of eDNA samples over time, a set of samples was collected from each of three water sources from the Croton River and Reservoir system (Fig I), one of NYC's sources of tap water. Specifically, we used five samples each from the New Croton Reservoir, the Black Rock Forest, and the Echo Boat Launch for a total of 15 samples. We then conducted two research ideas side-by-side: the potential of freezing the samples as well as the effect of storage time on the samples' precision in taxonomic identification. Each of the samples were filtered and the product stored at room temperature in lysis buffer from the Qiagen PowerWater DNA extraction kit; one sample from each of the three sites was stored in lysis buffer in the freezer at -20 oC and eDNA extraction completed 16 days after sampling. DNA extraction was completed for one sample from each of the three sites on the same day, as well as 4, 8, and 16 days after sampling.

After eDNA is extracted from each sample at the appropriate time, we quantified the DNA concentration from each sample using a Qubit 2.0 Fluorometer. We conducted PCR reactions for trnL and arthropod specific 16S. The samples were then prepared for gene sequencing by cleaning the PCR materials using ExoSAP-IT and then sequenced at GeneWiz using a MiSeq with 2 x 250 base pair reads. We then developed data-analytic scripts in R to interpret the NGS data, and assess the differences amongst samples containing eDNA under varying periods of time. Species composition was compared using ordination via the metaMDS in the R package Vegan.

Results

Freezing the samples had little impact on the quality or concentration of the extracted DNA.

As time went on, the DNA concentration slightly decreased (Fig II). However, the decrease is not dramatic enough to entail leaving the field to extract the DNA. We also found that freezing does not affect DNA concentration dramatically.

Furthermore, our research suggests that the sample composition did not vary over time (Fig III, Fig IV). For Fig III and Fig IV, the words at each point represent the location of the sample and the number is how many days we waited before extracting the DNA. Each circle represents a specific site. The data shows that generally the samples are closely clustered together for each location. Also, there is a minimal variance between the concentration of DNA from the same sampling site at different conditions.

Figure I: Sampling Sites along the Croton River and Croton Reservoir

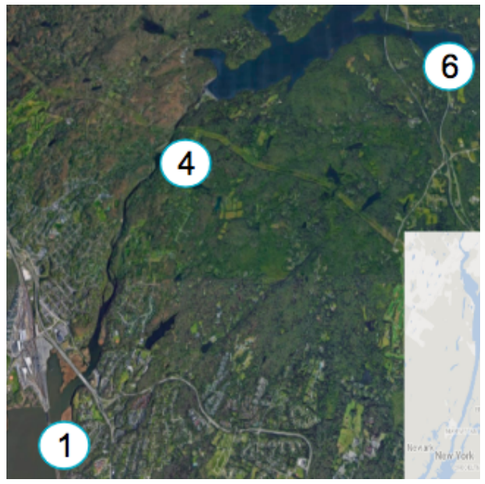


Figure II: Concentration of DNA samples over time

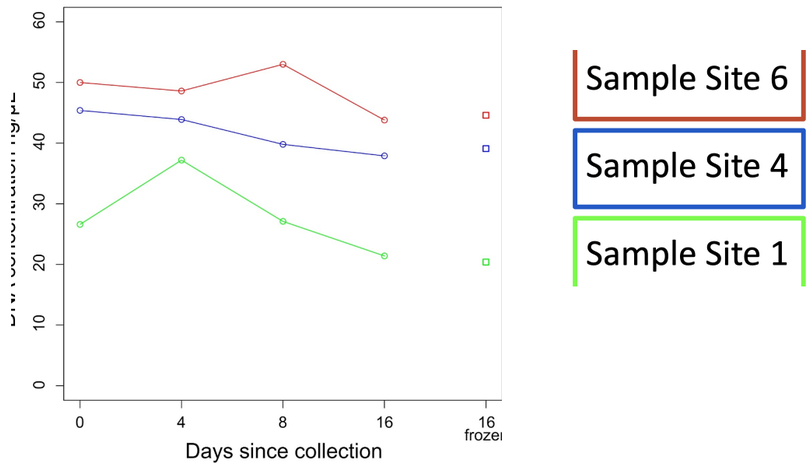


Figure III: Ordination Graph of trnL2 primer DNA viability

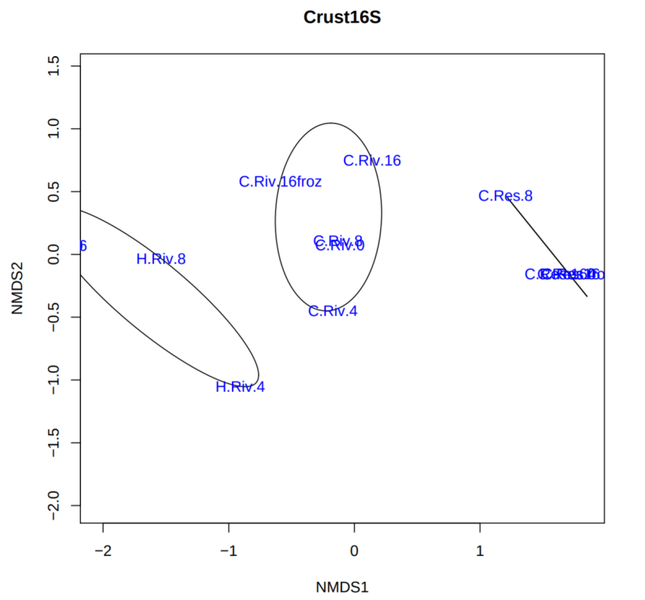
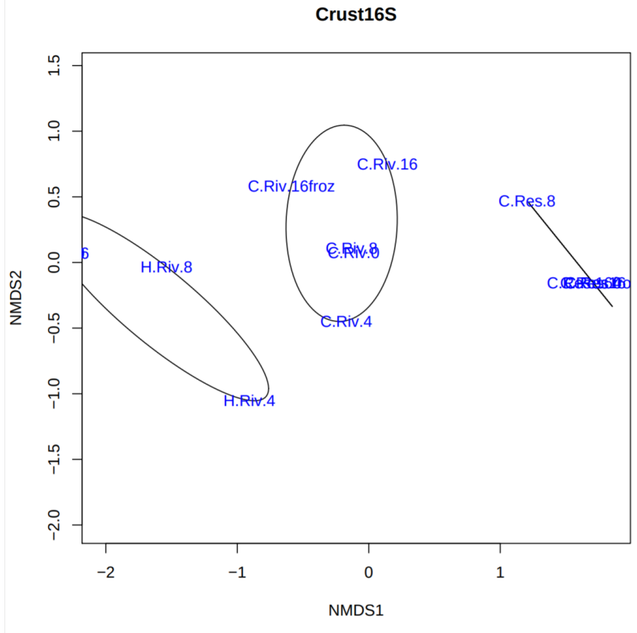


Figure IV: Ordination Graph of Crust16S primer DNA



Discussion

We conclude that temperature and the prolonged storage of DNA have minimal effects on the concentration, viability, and composition of the DNA sample. The results obtained in this study are significant because they highlight that DNA concentration minimally changes over time. This is significant because it means that researchers do not need to grapple with the issue of collecting samples and immediately leaving the field to extract the DNA. As a result, this study paves the path for future eDNA research and explorations with definitive understandings of how DNA concentration changes while in the laboratory.

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aqOAPorin-4: Investigating Array Formations in M1 and M23 Isoforms

Sherry Chen, Elizabeth Doss, Talia Kahan, Justin Lam, Nelson Liu, Leonard Ma, Kristoff Misquitta, Michael Nath, Lauren Pehlivanian, Benson Weng

Advised by: Dr. Tu

Abstract

Aquaporin is a protein that selectively transports water across membranes. Aquaporin-1, the most studied, functions across the membranes of erythrocytes and endothelial cells. In contrast, Aquaporin-4 is found primarily in star-shaped astrocytes of the blood-brain barrier and is responsible for regulating water homeostasis in the brain. Structurally, AQP4 is different from AQP1 due to variances in the Asn-Pro-Ala motifs in AQP4, which allow water molecules to move more freely through the channel.

AQP4 research is of much interest due to the importance of regulating cerebral water homeostasis. Even a slight increase in brain water levels leads to an increase in pressure within the brain and the compression of brain tissues. Therefore, any mutation in its structure will cause serious damages. For example, malfunctioning of AQP4 is linked with *neuromyelitis optica* (NMO), an autoimmune disease.

There are two isoforms of AQP4: M1 and M23. The brain expresses both isoforms, while skeletal muscle, the stomach, and lungs express predominantly M23. They differ in their translation initiation sites; in M1, translation starts at Met-1, whereas in M23, translation begins at Met-23. As a result, M1 is 22 amino acids longer and is referred to as the *long isoform*, while M23 is the *short isoform*. Due to the structural difference between the two, M1 diffuses freely through membranes, whereas M23 forms *orthogonal arrays of particles* (OAPs) that prevent easy diffusion.

Neuromyelitis Optica and Its Treatment

NMO is an autoimmune disease caused autoantibodies attacking AQP4. This leads to demyelination and eventual death of astrocytes, affecting the central nervous system. NMO often affects optic nerves, the spinal cord, and the brain, causing blindness and paralysis. In children, it can also cause confusion, seizure, and coma. Treatment development of NMO is not a contemporary research topic due to the rarity of the disease, but long term remission is possible and the availability of AQP4 antibodies has allowed for more accurate diagnosis.

Although anyone can contract it, repeat testing is recommended for females 39 years and older, who are nine times more likely to be diagnosed with NMO due to the low incidence of AQP4-IgG-positive NMO antibodies. In addition to the AQP4 antibody tests, further testing such as imaging tools should be applied to the patients. The different practical imaging tools in screening NMO are magnetic resonance imaging and optical coherence tomography.

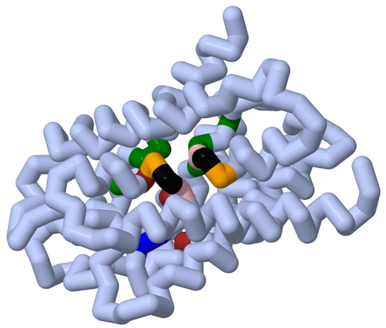
Despite these methods of detection, a curative treatment for NMO does not exist to date. Instead, patients with NMO must rely on treatment goals -- the remission and improvement of relapse-associated symptoms, the long-term stabilization of disease course through relapse prevention, and symptomatic therapy of residual symptoms. Detailed studies on the structure of AQP4 could facilitate the development of an effective treatment in the near future.

AQP1 vs AQP4

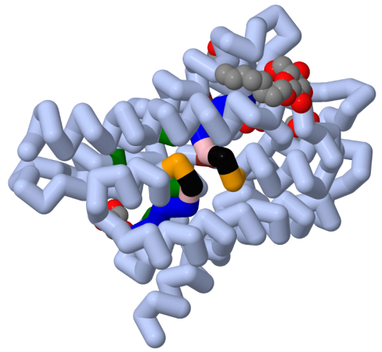
The Aquaporin family consists of 13 different types of proteins in humans. They are generally responsible for the conduction of small molecules, such as water and glycerol. Both AQP1 and AQP4 serve similar functions and can be found in the brain. However, while AQP1 can also be found in red blood cells and the renal proximal tubules of the kidney, AQP4 is largely reserved to the astrocytes that are in contact with the blood-brain barrier. On a structural level, both AQP1 and AQP4 are tetramers, which consist of four monomers. AQP4's monomers are surrounded by 6 alpha helices and 2 half-length alpha helices, and AQP1's monomers consist of six, slanted alpha helices that span the width of a membrane. In both proteins, the monomers form a four-fold axis. The pathway in both proteins' channels are amphipathic. For example, the channel for AQP4 has one hydrophobic side, lined with side chains of Phe-77, Ile-81, Val-85, Leu-170, Ile-174, and Val-197, and one hydrophilic side, formed by Gly-93, Gly-94, His-95, Ile-96, Gly-209, Ala-210, Ser-211, and Met-212.

At any given moment, the 8 backbone carbonyls that contribute to the hydrophilic side in AQP4 create 8 possible positions for water molecules in transit. This is because, in two of the Asn-Pro-Ala motifs of AQP4, Asparagine 213 and 97 each donate one hydrogen bond to a different water molecule. Since each asparagine can donate its hydrogen bond to a water molecule, water molecules may be freer to move in AQP4's channel. On the other hand, the two corresponding asparagines in AQP1 donate their hydrogen bonds to the same water molecule. Both AQP4 and AQP1 are able to prevent the conduction of protons because of the orientation of the water molecules inside the channel and the induced dipoles at regions of the Asn-Pro-Ala motif. In addition, unlike AQP1, AQP4 function is not inhibited by mercury because it lacks the corresponding Cys-191 residue found in AQP1.

Model of Aquaporin 1



Model of Aquaporin 4



Backbone carbonyl atoms

Hydrophobic linings

Helps exclude large solutes:
Left- Phe-56, His-180
Right- Phe-77, His-201

Asn-Pro-Ala motifs:

Left- Asn-716, Asn-192
Right- Asn-213, Asn-97

Left- Pro-77, Pro-193
Right- Pro-98, Pro-214

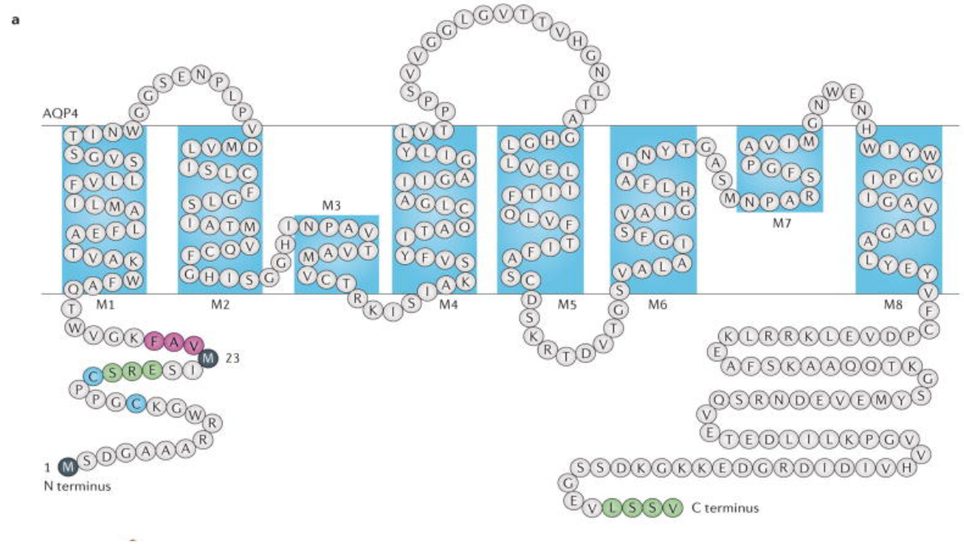
Left- Ala-78, Ala-194
Right- Ala-99, Ala-215

M1 vs M23

Alternative splicing of AQP4 mRNA results in two isoforms: M1 and M23, named by the methionine residue at which translation begins. M23 is 3000 times as abundant as M1 in the brain, and can induce OAP formation in other members of the AQP family. The change of just 22 amino acids significantly impacts the behavior of each variant within the cell membrane. M1 can rapidly diffuse along the spider-like processes of astrocytes, supporting cell movement. M23, however, due to its unique structural polarity, forms massive OAP aggregates, thought to boost membrane permeability. The OAPs created by M23 are too large to diffuse quickly across the cell membrane. They instead serve as anchors and bind to adhesion complexes of astrocyte endfeet. Each isoform thus, expresses a distinct cellular localization pattern. M1 is much more involved in cell migration, but M23 is much more involved in water transport across the blood-brain barrier. Furthermore, combining M1 and M23 in the membrane elicits an entirely different response: OAPs with M23-enriched cores encased in a layer of M1 isoforms take shape.

Each isoform's aggregation of OAPs of different sizes allows their respective astrocytes to carry out functions such as migration (M1 induced) and adhesion/polarization (M23 induced), without having to change water permeability. M1 and M23 isoforms, for the aforementioned events, are of interest when researching diseases such as NMO. NMO-IgGs do not have the ability to bind to a linear AQP4 epitope, but they are able to bind to the epitopes of AQP4-OAPs, notably ones created by AQP4-M23.

Models of M1, M23



Acknowledgements

We would like to thank Diane Munzenmaier and Tim Herman at the Milwaukee School of Engineering and Center for Biomolecular Modeling (CBM) for their introduction to the MAPS competition.

We would also like to give a special thanks to Mr. Suter for helping us print 3D models of the AQP molecule and to the biology and chemistry departments of Stuyvesant High School.

We would also like to thank Dr. Tu for for being so patient and mentoring us throughout this entire process.

Lastly, we would like to thank the Student Union and Parents Association for funding this project.