Research of White Sucker Cell lines and Associated Viruses

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Abstract

Catostomus commersonii, also known as the White Sucker, is a vital part of most aquatic ecosystems in the U.S. Specifically looking at the Upper Midwest, these fish are widely distributed around the states of Minnesota and Wisconsin. The importance of these organisms is due to their species reputation, they are indicator species that are very useful in informing whether the environment around them is healthy or if contamination has occurred. To better understand the organism, we needed to start at the molecular level. Understanding the nature of White Sucker cells through cell cultures. To get the individual fins of the White Sucker to flasks with growth media and begin to have the cells grow from the tissue. Through process of cell culturing, the cells differentiated from one another through each passage and adapted to different media to create contrasting growth rates. The differentiating cells were then characterized through DNA barcoding and examined for susceptibility to a variety of viruses to obtain better information about the multiple cell types from the fins of the White Sucker.

Introduction

The White Suckers is a very common fish throughout the state of Minnesota and other areas in the Upper Midwest. White Suckers have a long body with a brown completion on the sides and a white belly. The average size goes from 10-20 inches long and weigh between 1-2 pounds. Being part of the Sucker family of fish, this means that the fish's physiology is similar to other sucker. Most suckers including White Suckers, have there mouth on the bottom side of their body with large lips. This makes it easier to scavenge food at the bottom of lakes, rivers or ponds. The White suckers are Benthic (bottom dwellers), so there diet consists of anything found at the bottom of lakes, rivers or ponds. This includes plant matter, insect larvae, snails, algae, decaying matter and also fish eggs. White Suckers have little preference on where they live. It is a very common species of fish that doesn't seem to have trouble with harsh conditions that might render other fish from populating a certain environment. This includes polluted waterways that would eradicated some fish but not most bottom dwellers.

In recent new there has been an outbreak of White Sucker contracting a new novel Hepadnavirus in the Great Lake Region of the United States. This new hepadnavirus seems to be a hepatitis B-like virus with a similar effects as other hepadnavirus when infecting a host. This discovery shows that hepadnavirus that are known to only resort in infecting mammalian and avian species, might now have a new branch of the viral family that infects fish. So understanding the nature of this particular virus is important. The new group of viruses and the inter-workings, can be useful in understand the hepadnaviruses that infect humans and cause one of the deadliest diseases, hepatitis B.

As a part of understanding the organism better, we will need to investigate the White Sucker at the molecular level. To understand an organism at that level we'll need to have a cell line to be maintained in a laboratory setting. With that we will need to have cells from different types of tissue that can be susceptible to infection. The tissues that was used were fins and scales of the White Sucker. For a cell line that could possibly be infected with this new novel hepadnavirus, there will need to be a cell culture of liver cells. Unfortunately the two attempts to culture the liver cells from the fish were unsuccessful. On the contrary, the fins and scale cell lines began to grow eventually leaving only the cells from the fin to be used for our research project. Once the types of cells that will be used was determined, subculture was needed to have hopefully different types of cell from that individual tissue. As the earlier passages being the most diverse with different types of cells, to the new passages created that are closer to a homologous culture. With this we hope to further verify the cells through cell characterization. As of now we have two different morphological cells stemming from the parent flask WSPA. Hopefully we will have two different cell verified and begin virally infecting them to see if the results of the infection differ from cell types.

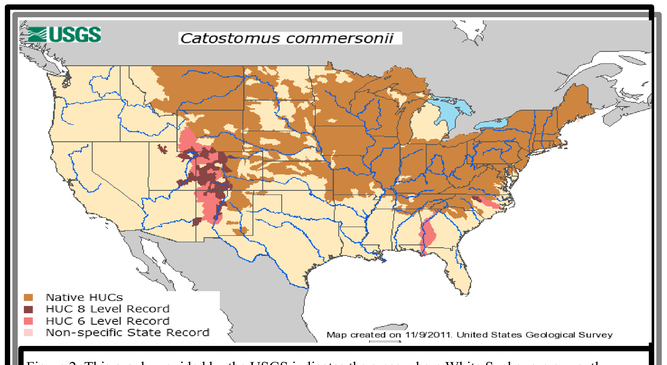


Figure 2: This graph provided by the USGS indicates the areas where White Sucker are currently located. The orange shaded areas are the places where the White Suckers are native. Minnesota and Wisconsin being shaded orange means that they are native to many of the waterways including the Mississippi River. The red shaded areas mean that the White Suckers were introduced there.

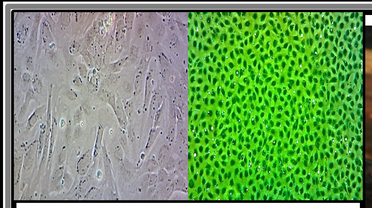


Figure 3: First cell line shown above with the WSPAD flask(left, 10X) compared to the WSPD-4 flask(right, 4X). The cells that have arisen from this line is epithelial cells.

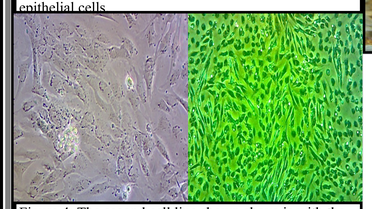


Figure 4: The second cell line shown above is with the WSPAD flask(left, 10X) compared to the WSPD6-1(right).

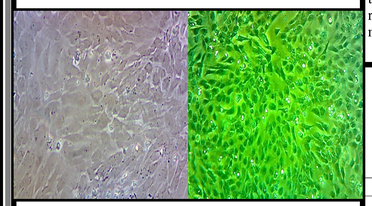
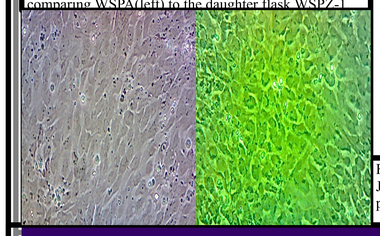


Figure 5: Looking at the third cell line from the parent flask WSPA(left) compared to todays WSPM-1(right) Figure 6(below): The fifth and most recent cell line comparing WSPA(left) to the daughter flask WSPZ-1



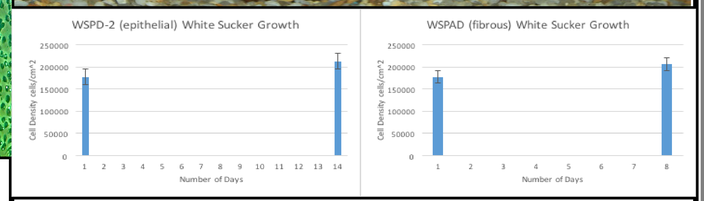


Figure 7: The graph on the left represents the growth rate of the epithelial cell in the WSPD-2 flask which is the parent flask of the WSPD-3/4 that are mainly epithelial cells. The graph on the right represents the growth rate of the fibrous cells. The growth rate of the epithelial cells is slower than the fibrous by 6-7 days. This might be due to the cell contrasting growth .

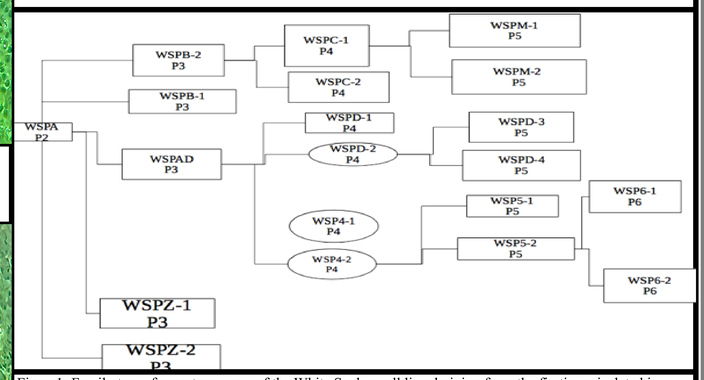


Figure1: Family tree of recent passages of the White Sucker cell line deriving from the fin tissue isolated in June 30th, 2017. With the 4 separate lines that were created, 2 distinctly cells (fibrous and epithelial) from the parent flask WSPA P2.

Methods

Isolating the tissue

Obtaining the White Suckers from the bait store and transporting them to the Fish and Wildlife center. This is where the procedure took place to extract tissue from the organism to flasks with media. The plan was to have 6 T-25 flasks (3 for fins and 3 for scales) with MEM10/20 serum and no enzyme treatment. The serum contained 1% gentonysin, 1% nistatine. Grabbing a sucker from the beaker(euthanized) and slicing the fins. Placed the fins in media outside of the flask and began to slice into small pieces. Three T-25 flask were made each having one Sucker. Then place tissue into appropriate flasks and incubate till 100% confluence has occurred. This whole procedure took place at the Fish and Wildlife Center in LaCrosse, WI.

Subculture

Procedure for subculturing the White Sucker cells was crucial to carry on the life of the cells from the extracted tissue of the organism, being the fins. This was only done once the flask has reach a 100% confluence meaning that the cells have spread around the entire surface of the T-25 flasks that they were placed in. The flask need old spent media to be removed and the addition of 2 mL of DPBS (Dulbecco's phosphate-buffered saline) which is a salt solution to help was the cells before being transported to another flask. Swirling the flask for 2-3 minutes and removing the waste immediately. Trypsin is used to make the cell detach from the bottom surface of the flask. Trypsin itself is a proteolytic enzyme used to break down the protein that make cells no longer attached to the flask. The trypsin usually is place and swirled around for 5 minutes, if cells are still adherent then either more trypsin should be added or longer waiting period for the cells loss it adherence. Only 1 mL of trypsin was need for the White Sucker fibrous and epithelial cells. The flask might need some taps on the side to contribute to the cells losing its grip of the flask. While the cells are being trypsinized, the set-up of the two new flasks (T-25) labelled and with 3 mL of fresh media (MEM20/MEM10) placed in to have the flasks prepared for the transport of cells. Once the cells have been in trypsin for 5-6 minutes, the addition of 5 mL of media (MEM20/MEM10) was placed in the flask with the cells for a total volume of 6 mL . The ratio agreed upon for the parent flask and the daughter flask was a 1:3 split, meaning that 2 mL of cells/media/trypsin would be placed in the new daughter flasks. Each flask will contain 2 mL or 1/3 of the total cells that were adherent to the parent flask.

Results and Discussion

-Figure 1 shows the lineage of the passages produced during the duration of this research. The tree doesn't show the flasks produced before the start of subculturing due to there being only 3 flasks produced on the 30th of June. Starting off with 3 flasks for White Sucker fin and 3 for the scales. Unfortunately the cells on the scales never were able to reach the growth rate to be subcultured into many different flasks. With a simple 1:3 split creating two new flasks from the parent flask this process was able to develop 4 different lines from the original flask WSPA. As seen on the tree, WSPA is a second passage cell line with its parents being from the original flasks that were produced down in La Crosse with the help of the Fish and Wildlife Center.

Cell Lines

-The first cell line was produced through the WSPAD flask creating the WSPD-1/2 being produced on 27th of November, only 7 days after seeding the flask itself. The next subculture for the WSPD-2 was done on the 11th of December to produce WSPD-3/4. This first cell line was the main line that differentiated from the rest due to its growth of epithelial cells (figure 3). The characteristics of epithelial cells include round structure unlike the fibrous tissue which are long and stretched out, nucleus more centralized than other cells and they fit together differently compared to the fibrous tissue.

-The second cell line produced was through the WSPAD passage 3 flask just like with the first cell line. The new flask produced were WSP4-1/2 on the February 3rd resulting on the new fourth passage cell lines that was subcultured again to produce WSP5-1/2. It also had another similarity with the first cell line created, WSP5-1 flask was reported to have a mixture of epithelial cells and fibrous cells seen on figure (4).

-The third cell line came from the WSPA flask unlike the first or second cell lines. It produced the WSPB-1/2 on the 20th of February. This meant that the WSPA flask wasn't subcultured sense the creation of the WSPAD flask on the 27th of November. This was done with routine refeedings so that the cells would be able to survive. One problem was that they would need more room so it seemed like a good idea to have the third cell line derive from the WSPA instead of the WSPAD. This cell line is mainly composed of fibrous tissue unlike the first and second. Cells characteristics include elongated structure, nucleus not really in the "center" of the cell and rather quick growth rate compared to the epithelial cells (figure 5).

-The fourth and final cell line only has two flasks from the WSPA flask (when the figure from the tree was created but as of April there are 4 flasks produced). The subculture took place on the 15th of March with the creation of WSPZ-1/2. These two flasks don't seem to have any epithelial cells growing within them making them solely fibrous cells (figure 6).

Growth Bar Graphs

- Looking at the bar graph that were produced (figure 7), there needed to be two different graphs for the two different morphological cells discovered in the cell line. When seeding, the count is read around the lag phase of growth. The seeding density should be smaller than the density read when the flask is ready to be subcultured due to the growth of the cell during it time in the flask.
-For the epithelial cells taken from the cell counts from the WSPD-2 flask, the initial seeding density was 177,760 cells/cm^2 and the final cell density was 205,920 cells per cm^2.
-For the fibrous cells taken from the cell counts from the WSPAD flask. This flask was the parent flask for 2 out of the 4 cell lines produced. The other 2 lines were from the WSPA flask which is the parent of the WSPAD flask.
-Only two points were read due to the restricted access of only reading the cell counts during subculturing. We could risk producing flasks for just cell counts because these cell were our primary source to infect viruses and conduct cell characterization. The growth rate also effected the cells as change media from MEM20 to MEM10 rendering the accelerated growth.

Virus Susceptibility

-Hopefully we will be infecting the different cell types from the fin of the White Sucker from an array of viruses supplied by the Fish and Wildlife Center.

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WSPA
P2

WSPB-2
P3

WSPB-1
P3

WSPC-1
P4

WSPC-2
P4

WSPM-1
P5

WSPM-2
P5

WSPAD
P3

WSPD-1
P4

WSPD-2
P4

WSPD-3
P5

WSPD-4
P5

WSP4-1
P4

WSP4-2
P4

WSP5-1
P5

WSP5-2
P5

WSP6-1
P6

WSP6-2
P6

WSPZ-1
P3

WSPZ-2
P3