Joseph Lancman, Ph.D. Project Updates

Update on 6-31-18

It's hard to believe that we have been at this for 1 year! We have accomplished a lot and have definitely taken major steps towards achieving our goal of converting other cell types, like muscle cells, into beta cells while they remain in the body. Over the past year, we have optimized our protocol for specifically collecting reprogrammed cells from an animal model and we have also dramatically increased the efficiency of reprogramming by using modified factors. With this work, we have positioned ourselves to utilize cutting edge technology to thoroughly investigate the molecular changes that are occurring after we deliver our reprogramming factors into muscle cells in vivo. Armed with this information, we will be able to determine what other factors or chemicals we can add to further coax muscle into a beta-like cell. From your front row seat over the last year, I hope you have come to appreciate how hard this type of work is and how important it is to be able to overcome roadblocks. In research, things rarely go as planned. We had many problems trying to collect enough reprogrammed muscle cells for in-depth molecular analysis. But we found ways to solve that problem and by doing so we have actually gotten better at reprogramming muscle cells in vivo. I think it is fair to ask what you have gotten for your donation. And I can say that your support was instrumental for the publication of our work. This might seem less than satisfying, but remember, once this work is seen by other researchers, many will repeat the work and try to further improve it. So, in a very real sense, you have amplified the number of people working on this approach and helped to inspire other really smart scientists to try and make it work. Moreover, by publishing this work, new funding opportunities should be available to for us which will allow us to dedicate more time and effort ourselves! This is a really great outcome and we should all be proud of what we have accomplished in this short amount of time. This has been a great learning experience for all of us and I wanted to take a moment to thank you for your support through the DRC. Without your support, this type of risky work would not have been possible and we would still be at the starting line wondering if this approach is even possible. As we move closer to publication, I would like to keep you informed of the review and publication process. Sometimes, this review and publication process can be quite lengthy. This is because, while we know how important this work is, we are trying to convince other experts in this area of biology of its potential and that our experiments were performed properly and the data we obtained is repeatable. This is how the review process works and is in place to prevent people from publishing work that is either not real or unrepeatable. So please keep looking for my updates as we obtain our new data and put together our scientific manuscript for publication. And remember, as I mentioned last month as well, publishing this work is a significant step as it legitimizes the work we have produced. So thank you again for all your support and your support of the DRC. And hang on for my updates as we move this work towards publication!

Update on 4-31-18

Well, that month went by quickly! We have been working out the kinks in our protocols and have had some good success isolating in vivo reprogramed muscle cells. As we continue to collect cells and settle on our analysis, I wanted to remind you of one of the potential advantages of our approach to eventually make beta cells within the body. As you may be aware, scientists

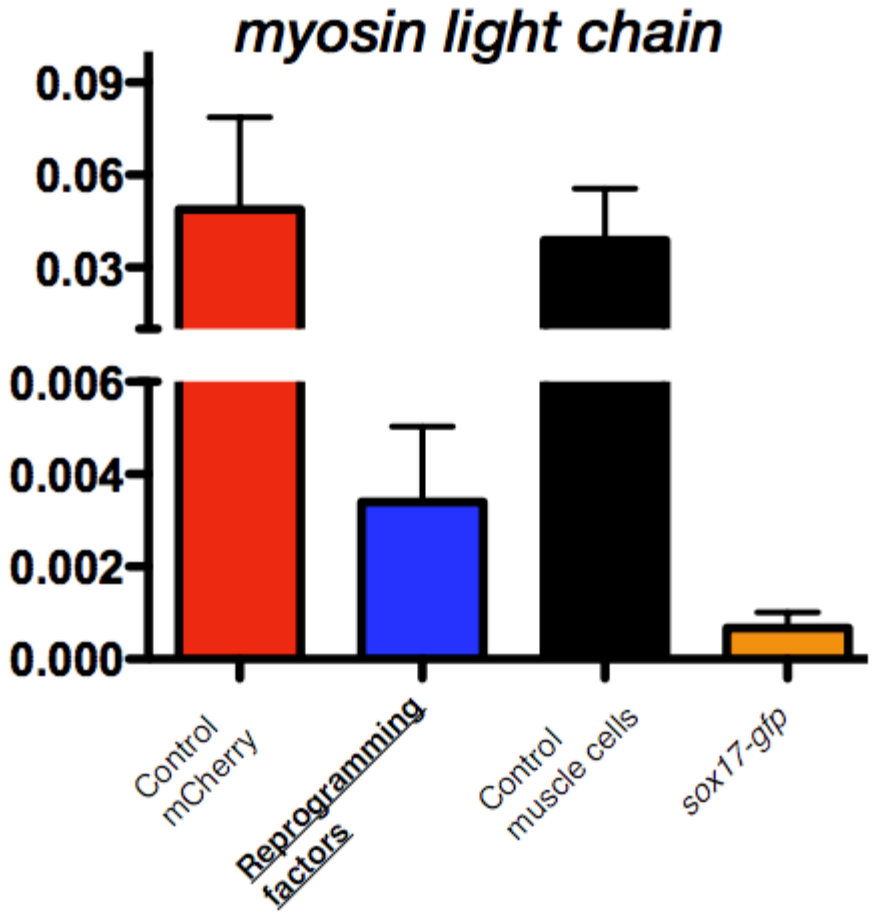
have created beta-like cells in culture starting with stem cells. The idea is to then transplant these lab-grown beta-like cells into patients with diabetes to replace the beta cells that have been lost or damaged. But there are two significant problems with this approach. First, we know that you can't just put these reprogrammed beta-like cells into the body because the immune system will recognize them as foreign and attack them. Second, because these cells are allowed to grow and divide many times while in culture, some may have acquired mutations that can make them unhealthy or even dangerous-having increased potential to be tumorigenic. A key reason that lab-grown beta-like cells can become dangerous is that in order to make them in the lab, one must first force a starting pool of cells into pluripotency. It is critical that the starting cells become pluripotent because, only pluripotent cells can be effectively guided to become virtually any cell type in the body, including beta-like cells. But this process is inefficient and requires the cells to divide many times. Therefore, while forcing cells to pluripotency and then guiding them into beta-like cells, mutations can occur and make the cell unhealthy or potentially tumorigenic. One advantage of the approach we are taking is that we avoid going through pluripotency. We predict that this will significantly reduce the chance that the cells we reprogram will become dangerous. Furthermore, because the reprogramed cells remain in the body, there are natural mechanisms that can help eliminate dangerous cells. But how do we know that the muscle cells we are reprogramming don't become pluripotent or pass through a pluripotent state? Fortunately, a lot of work has be done to determine the molecular changes required for a cell to become pluripotent. Therefore, we can use this work to ask if any of the characterized molecular changes required for pluripotency occur in our reprogrammed muscle cells. Again, this is why we want to isolate many reprogrammed cells. We want to isolate as many as we can so we can determine whether there are any molecular changes that are occurring that would make these reprogrammed cells dangerous. One of the key transcription factors that is necessary to reprogram a cell to pluripotency is called Oct4. In fact, this is one the 4 factors that Shinya Yamanaka found, and won the Nobel prize for, to be necessary to reprogram almost any cell into a pluripotent cell in culture. Because of how critical Oct4 is for inducing pluripotency, we looked at whether the muscle cells we reprogrammed turned on Oct4 at any time. And so far, we have not detected up-regulation of Oct4 mRNA in reprogrammed muscle cells! This is a good piece of evidence that in vivo reprogrammed muscle cells do not become pluripotent. But there are other molecular hallmarks of pluripotency that we must still check, once we isolate enough cells for analysis. What this ultimately means is that in vivo reprogramming has the potential to be safer than other methods that are used to make beta-like cells and require the starting pool of cells to be pluripotent.

Update on 3-31-18

Over the past month, we have been making solid progress with our project. We are now working out the experimental conditions to properly isolate our reprogrammed muscle cells so we can perform in-depth molecular characterization. The details of this work are pretty technical and probably not that interesting to a majority of you. So I am just going to include some data that we are using to validate that our reprogramming and muscle cell isolations are working. What I have included below is a chart that shows the expression levels of a transcript called myosin light chain. This transcript is important for muscle cell function and is not highly expressed in the tissue that normally gives rise to the pancreas and beta cells. In the chart below, each bar represents how much of myosin light chain transcript is expressed in the different populations of

sorted cells. For example, the red bar is the amount of myosin light chain expressed in isolated muscle cells that have received a control protein-mCherry. The expression level in these muscle cells is very high. In fact, it is identical to the black bar, which represents the amount of myosin light chain detected in normal, isolated muscle cells. This is what we would expect. Now, if you compare the amount of myosin light chain expressed in either the mCherry control muscle cells (red bar) or normal muscle cells (black bar) to muscle cells from the blue bar, you can see there is a lot less myosin light chain expressed in cells isolated for the blue bar. The blue bar represents the muscle cells that we have reprogrammed! So you can see, once muscle cells that receive our factors begin to change their identity, they also begin to lose factors that are important for their original muscle cell identity/function. This is a good sign as cells that normally give rise to the pancreas and beta cells only express very low levels of myosin light chain. This can be seen by looking at the last bar on the chart (orange), which shows the amount of myosin light chain expressed in cells isolated from the tissue that normally gives rise to the pancreas and beta cells. There is hardly any myosin light chain expressed in these cells. Interestingly, if you now compare the blue bar to the orange bar, you can see that the reprogrammed muscle cells express more myosin light chain and therefore are not completely behaving like the cells that give rise to the pancreas and beta cells. What we hope to discover is why! This will help us to determine how to keep pushing the reprogramed muscle cells towards a more authentic beta cell identity! This analysis was done on only one transcript-myosin light chain. Ideally, we will characterize all the transcripts in isolated cells (reprogrammed and control) to get a comprehensive view and understanding of the molecular changes that occur in reprogrammed muscle cells. So hold on as we can continue to look at the changes from many more transcripts!

Muscle identity is lost in FACS sorted muscle cells undergoing lineage reprogramming



(Image and data courtesy of Clyde Campbell)

Update on 3-06-18

Over the past few months, we have been resolved to optimize our isolation of reprogrammed muscle cells so we can really start to understand what kind of molecular changes are occurring as the muscle cells change identity in vivo. With this in-depth understanding, we think we will be better able to guide the reprogrammed muscle cells further along towards an early pancreatic cell identity and ultimately a beta-like cell identity.

And after several months, it is with great pleasure that I am excited to let you know that we have finally (finally!) overcome our technical problems! We were finally able to successfully isolate many reprogrammed muscle cells following our experiment! With this advance, we can now

combine isolated reprogrammed muscle cells from 2 or 3 experiments and perform the in-depth molecular analysis we had originally proposed to do.

Because of the problems we were having isolating large numbers of reprogrammed muscle cells, I mentioned in my last update that we began pursuing another type of analysis that did not require us to isolate so many reprogrammed muscle cells. This alternative approach allows us to isolate individual reprogrammed cells, and perform analysis on each separately, rather than analysis on a large pool of cells, like our other technique. After having discussed our experimental details with experts in this single cell analysis technique, we have concluded that this alternative strategy to analyze individual reprogrammed muscle cells will also be feasible!

One month ago, we were stalled as we attempted to isolate enough reprogrammed muscle cells for in-depth analysis. But over the past month, we have found TWO viable ways to perform in-depth molecular analysis of in vivo reprogrammed muscle cells! This is really exciting and shows you how fast our fortunes can change in science when we continue to push and work hard. This is a major step for us as we continue to pursue our goal of in vivo reprogramming of cell identity towards early endoderm identity and eventually beta cell identity!

Thank you for hanging in there with us. It's been a bumpy ride. But we will continue to make progress and push forward!!!

Until next month……..

<u>Update on 11-30-17</u>

With the holiday season upon us, it's a good time to step back and reflect on our past year and be thankful for all the positive events we have been associated with. This also applies to science, especially when things aren't going as well as we'd like. If you recall, our goal for the previous month was to try and continue to optimize the number of reprogrammed muscle cells we collect. This is because we had issues collecting enough reprogrammed cells to perform in-depth analysis of the changes that are occurring within these reprogrammed muscle cells.

Unfortunately, we have not yet been able to improve our yields of reprogrammed cells. But we are continuing to work hard to improve our outcomes. Importantly, these challenges we have encountered have provided an opportunity to try some new ideas on how to increase the number of reprogrammed cells we are getting. One of which I'd like to share with you. As you know, we use two factors to change the identity of muscle cells into early endoderm-like cells, the cells that can normally go on to form beta cells in the body. These factors we use are a special type of protein called a transcription factor. Transcription factors are primarily found inside a cell's nucleus where they can turn genes on and off.

One of our ideas was to try and make the transcription factors we are using better at turning on the genes needed for early endoderm identity. What is really amazing is that many other researchers have modified transcription factors to make them more active or better at what they normally do. In a sense, they are like super versions of themselves! After looking at how others have made transcription factors better, more potent, we have decided on a few changes that may

enhance each of the transcription factors we use. To make the changes, we needed to either add new parts or change specific sequences in the mRNA of the transcription factors. We have completed the changes to one of our factors and are now working on making changes to the other. Once we make the changes to both, we can test them and determine whether they enhance reprograming in vivo.

We are excited about this approach and believe it could be an exciting new way to enhance the reprogramming that we are trying to accomplish in vivo. It should also help us collect more reprogrammed cells in order to carry out the analysis we would like to perform. We also believe that, ultimately, by having more reprogrammed cells to start with, we will have more opportunities, and therefore a better chance, to push their identity closer to beta-cells.

So enjoy the holiday season and keep in mind that we are working hard to fix our technical problems. The sooner we can clear this hurdle, the sooner we can continue to try and push reprogrammed cells towards a beta-cell identity in vivo!

Update on 10-31-17

Sometimes in research (often!), things don't go quite as planned. The past month has been a challenge in regards to our research efforts and as a result, our progress has been slowed.

Our goal for the past month was to try and continue to optimize the number of reprogrammed muscle cells we can collect in order to do in-depth analysis on the changes that are occurring within these cells. Recall that we are trying to look at all the changes that occur in mRNA expression levels in reprogrammed muscle cells compared to normal, control muscle cells that haven't been reprogrammed. By analyzing the changes, we can get a better idea of what else we need to do to push the identity of reprogrammed muscle cells closer to a beta cell identity.

Because each cell we collect only gives us a tiny amount of mRNA, we need to collect many, many cells in order to gather enough mRNA to do in-depth analysis. Unfortunately, we still haven't been able to increase the number of reprogrammed muscle cells that we can collect at a single time. So, we are generating new ideas and are still confident will be able to generate and collect the larger numbers of reprogrammed muscle cells we need to carry out our analysis.

We believe that most of our problems are stemming from how we are delivering the reprogramming factors. The most likely problem is that we are not getting enough into the cells. What's interesting is that we can still learn a lot about the biology of reprogramming from the problems we encounter.

So what can we learn from our current problems? It seems that the amount of reprogramming factors we are using and putting into the cells may be a critical parameter for success. Too little, and reprogramming doesn't occur at high enough incidence. On the other hand, putting in too much of the reprogramming factors might also reduce our reprogramming efficiency. We can say this because those performing reprogramming of cells in vitro have already documented this phenomenon. Therefore, we can tentatively infer that, like those working to reprogram cells in

vitro, we need to be careful to add just the right amount of reprogramming factors to achieve optimal levels of reprogramming.

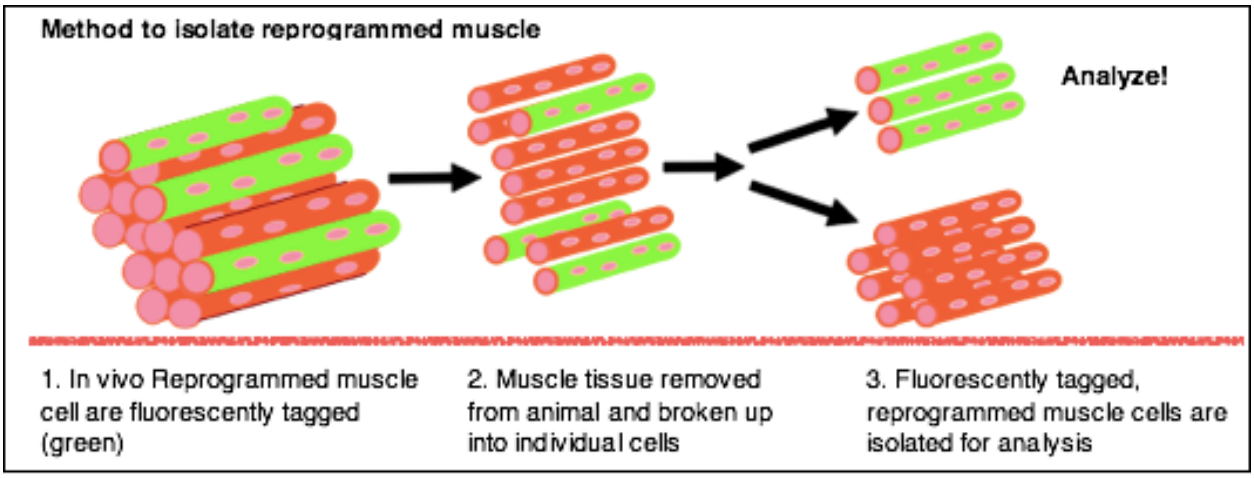
More importantly, because we are experiencing some of the same problems people working on in vitro cell reprogramming have experienced, it means we can learn a lot from them and apply it to our problem(s)! So don't get discouraged. These delays always occur in research. With hard work and creative problem solving, we'll get through it. Stay tuned as we continue to solve our technical problems and keep pushing forward….

Update on 9-30-17

Hello! It's time again to give you all a quick update on our progress over the past month. As I mentioned in my last update, we were working hard to iron out some of the technical problems we were having as we attempted to isolate reprogrammed muscle cells that were changing their identity.

The reason we need to isolate these reprogrammed muscle cells is so we can carefully analyze and characterize how their identity change is occurring. Armed with that information, we can then determine what other changes we need to make in order to continue to push reprogrammed muscle cells into beta cells.

Below is a cartoon to remind you what we are trying to do:



A key part of our experiments that I think is important for you to understand is our need to also isolate and analyze control muscle cells. Control muscle cells are important because they do NOT undergo reprogramming and therefore tell us what mRNA normal muscle cells usually express. Knowing this, we can then compare the types of mRNA that normal muscle cells express to the mRNA that reprogrammed muscle cells express, allowing us to detect differences in mRNA expression between the two.

Over the past month we have repeated our experiments several times and tweaked several parameters in order to increase the number of reprogrammed and control muscle cells. This allowed us to isolate more reprogrammed or control muscle cells per experiment. Even though

we were able to collect more cells, we still haven't been able to collect enough cells to do really in depth analysis. However, we were able to isolate enough reprogrammed and control muscle cells to do important preliminary analysis.

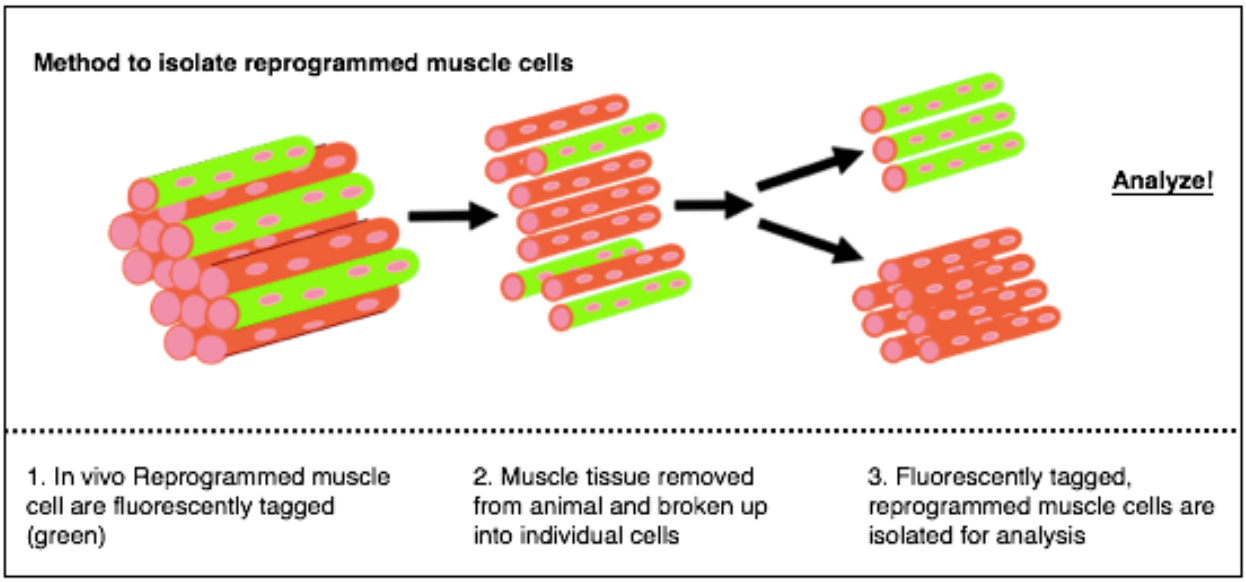
In our preliminary analysis of the molecular changes in reprogrammed muscle cells, we could detect expression of mRNAs that are only expressed in early naive gut like cells! Importantly, we never detected these mRNA in control muscle cells! This means that our analysis will work and that reprogrammed muscle cells are in fact changing their identity and beginning to express mRNA that is important for formation of early naive gut like cells.

So, for the next month, we will continue to tweak our experimental conditions in order to get more reprogrammed muscle cells and control muscle cells. This will allow us to do more in depth and complete analysis of the molecular changes that are occurring during muscle cell reprogramming.

More cells! More analysis! Until next month…...

Update on 8-31-17

The ultimate goal is to determine whether we can transform completely unrelated cells in the body into functional beta-cells by successfully reprogramming muscle or skin cells into a naive gut like cell that can go on to form tissue like the pancreas (where beta cells normally develop). We need to carefully analyze and characterize how this change is occurring so we can determine what other changes need to be made in order to continue pushing these reprogrammed muscle or skin cells into beta cells. Going forward, we will initially focus our efforts on reprogramming and analyzing muscle cells in order to thoroughly understand the molecular changes that occur while reprogrammed muscle cells change identity in vivo, this requires isolation of the reprogrammed muscle cells by separating them from muscle cells that have not been reprogrammed (see Diagram below).



Over the past month, we have made great progress in working out the proper conditions to efficiently deliver our conversion factors and the fluorescent tag to be sure that a muscle cell always receives both the factors and the fluorescent tag and still remains healthy. We also had to be sure that the fluorescent tag itself did not influence our reprogramming efforts (it does not). Although not fully optimized, we have had enough success to begin trial runs of cell sorting using FACS.

Over the next month, we will continue to optimize the delivery of the fluorescent tag and the cell sorting. During this time, the goal is to isolate enough reprogrammed muscle cells in order to begin in depth analysis of the molecular changes that are occurring. Armed with this information, we can begin making plans for how to try to keep pushing these cells closer to a functional beta cell!"

Update on 7-1-17

We have cleared a major hurdle in our efforts to convert completely unrelated cells in the body into beta-cell pre-cursors. We have discovered that delivering two factors into muscle or skin cells can convert them into a naive gut cell type that can go on to form tissue like the pancreas, where beta cells normally develop. In order to now try and continue to push these reprogrammed muscle or skin cells into beta cells, we need to thoroughly understand the molecular process that occurs while they change identity.

Over the next 3-4 months, I will be analyzing converted cells in order to determine the critical molecular changes that occur as they change their identity. These experiments will yield data necessary to inform our next strategy to drive these cells closer to becoming functional beta cells.

By successfully moving this project forward, we are exploring a fundamentally novel therapeutic approach for beta-cell replacement. It is also creating an opportunity to help those who deal with the complications of diabetes every day of their life. The experiments I am currently focused on should lead to new and exciting insights into changing cell identity in vivo and bring us a few steps closer to our goal of generating beta-cells in the body. And it is because of your generous support and the support of the DRC that it is possible.