Final Assignment

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Reviewing PNAS article titled

"A single combination gene therapy treats multiple age-related diseases"

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1 Personal motivation for choosing this paper

For this final assignment, I've chosen to review this paper called "A single combination gene therapy treats multiple age-related diseases" since the addressed problem is very impactfull. By only reading the abstract, I've noticed some interesting ideas such as how the majority of people nowadays suffer from multiple diseases which finally causes their death. In previous times, people used to have shortened lifespans, and their death was caused mostly by one fatal disease. Now, since we live longer we tend to "accumulate" multiple diseases and the treatments we are given usually target an individual disease, without taking into account the effects that one treatment could have for the other diseases. Furthermore, by using multiple treatments, the patient risks even more side-effects.

Another aspect that draws my attention is the age-related diseases that they've chosen in this article which are widely spread and some of the most relevant ones for this subject: obesity, type II diabetes, but also the heart and renal failure.

2 Context

As I've previously mentioned, this article focuses on treating 4 major age-related diseases by trying to find a gene combination that would actually show promising results for

all of them. The type of treatment that they were seeking is a gene therapy one in which they combined three genes known to be associated with longevity: the first one is FGF21, which stands for fibroblast growth factor 21, αKlotho and sTGFβR2. During this article, there were run multiple experiments on adult non-transgenic mice for each of the previously mentioned analyzed diseases by using the three AAV-based therapies either individually or combined. After making all these experiments, they've concluded that gene therapy combining the first and the second gene was the most effective. Also, by proving that there's such combination that can target multiple diseases they've demonstrated that gene therapy may also improve one's health span (which is defined as the period of time in one's life when one has no medical condition).

3 Introductory concepts

In order to move on and discuss the actual experiments, I'll start by presenting a couple of concepts. The first one is known as gene therapy, but what exactly does it mean? In general, gene therapy is an experimental approach that may, later on, replace the traditional treatments such as medication which is supposed to either alter a gene (meaning that a gene can be turned off), replace one or introduce a new one. By doing such things, one may treat certain diseases or even more, prevent them.

Another concept worth explaining is the AAV. AAV stands for adeno-associated virus and is a small in length, inoffensive virus since there are no known diseases caused by it. Also, the AAV can infect both humans and other primate species and is known to be universally distributed among the human population, but it is replication-defective (meaning that it needs help replicating) and nonenveloped (there is no outer protective layer). It was used so far in more than 100 medical trials and it is known for its contribution in gene therapy and transgenic (mostly for gene delivery). Since it is not able to replicate on its own, it needs a co-infector, also known as a helper, to trigger even a small immune response, but even together with that co-infector, there are no known causes diseases. When the AAV is firstly introduced, it camouflages in a small region of the 19th chromosome, and it stays in a dormant state until the helper, the actual adenovirus comes along. In this paper, there

were created three AAV8 vectors, each one for a specific gene and the number that follows the AAV is the encoding for the specific body region that it affects. That number is known as the serotype and until now there are know 11 distinct serotypes, from which numbers 2, 3, 5, 6 were found in human cells while the others in other primate species. Since these experiments where run on mice, it was used AAV8 which is also known for targeting and infecting the liver.

Regarding the four chosen age-related diseases, the article emphasizes the high number of patients affected by each one of them. Starting with obesity, which is known to affect more than a third of American adults, it affected more than 12% of the total worldwide population. Moreover, obesity and overweight were associated with almost 20% of deaths in American adults and is also linked to several other conditions such as heart diseases, stroke, diabetes, and breathing problems. The second condition mentioned in this article is type II diabetes, a condition that becomes more and more popular among all of us. In our days, it is frequent to meet an elder with diabetes type II, than one without it. According to World Health Organization, in 2016 there were more than 1.5 million deaths directly associated with diabetes and even more associated with diseases linked or caused by diabetes, such as heart attacks, strokes, or kidney failure. The last two conditions are heart failure and renal failure. It is known that heart failure is strongly connected to renal failure. Kidney (or renal) failure is another widely spread condition in the United States since according to their numbers, more than 600000 people need annually either dialysis (a procedure which basically eliminates impurities such as toxins and solutes out of one's blood, by getting the blood out of a patient, cleaning it up and then introducing it back again) or a kidney transplant. The last age-related disease mentioned in this paper is heart failure. They motivate this choice by emphasizing the similarities between the human heart and mouse heart in some aspects, while this condition is also widely spread in the United States as well, with more than 400000 death per year associated directly with this. Heart failure is not solely represented by having a heart attack, but it is mostly represented by patients whom hearts can no longer pump at 100% capacity. Even more, all of these previously mentioned conditions that have been chosen in the paper can be the ones to cause the appearance of additional medical conditions.

In order to conclude, I find that addressing the age-related diseases, in particular, these

four ones are a great starting point since any one of them could, later on, cause more and more damage by triggering other medical conditions. Thus, it is important to extend as much as we can one's healthspan.

4 Actual contribution

Moving on to the actual content of this paper, we will start by addressing the first experiment presented. As I've previously mentioned, obesity strikes strongly in the United States but worldwide as well. Moreover, obesity is the one that may trigger adjacent diseases as the one previously mentioned like cancer, neurological deterioration, but it may also impact the heart since it is now surrounded by fat which makes it harder and harder to pump. For the first phase of the obesity experiment, they've considered adult non-transgenic mice separated in 9 categories of mice: there are control mice fed a high-fat diet, control mice fed a normal diet (and both the control groups have been injected an AAV:GFP – which stands for green fluorescent protein) and also the mice used for administrating all the other possible combination of those 3 genes were kept on a high-fat diet during the time frame of the experiment, but also a period of three months before the experiment and three months after it. Mice fed with a high-fat diet before the experiment increased in weight with about 56% of their initial weight. Since starting the experiment, results started to show after the first month after the injection and the best results were noticed for AAV with FGF21 gene, but also for every other combination that this gene had with the other two. The results managed to be maintained and stay consistent over the 3 months of the experiment, but the researchers looked even further and noticed that there was no reverse to the initial mice weight even 8 months after when mice were still on a high-fat diet.

The one presented above was only the first part of the obesity experiment which was done on adult mice. Moving on, the same experiment was also done for aged mice (18 months old, which is the equivalent of 60-year-old humans) fed a normal diet, since the article emphasizes how older people, in general, tend to have an increased fat mass. In this case, we only had control mice and mice injected with one of the 8 possible gene therapy combinations (either an individual gene, in a combination of two or all three of them). In

comparison to middle-aged mice, in this age group not only the FGF21 combinations were successful in reducing mice weight, but also all the αKlotho combinations showed a decrease in weight and all of there results managed to be maintained for the next 250 observation days.

To better analyze this phenomenon and see if the gene therapy was actually effective, the middle-aged mice fed a high-fat diet, which were injected only with the AAV:FGF21 gene therapy were placed in special chambers for monitoring their whole metabolism (their food intake, their CO_2 emissions and so on). When monitored, there was noticed an increase in both the O_2 intake and CO_2 emissions, meaning that their metabolism actually speeds up, in comparison to the control mice which were fed with a high-fat diet, but were not injected with AAV:FGF21. Their respiration also became more regulated and their food intake suffered no changes, so both the control mice and the treated mice with AAV:FGF21 were consuming the same amount of food, but their differences in corporal mass were coming from their changes in metabolism. Studies were also conducted in order to see if the muscle mass was affected, but it was noticed that the change in weight came solely from fat loss.

Moving on to the second analyzed condition, which is type II diabetes, the same mice and categories were kept, but there were analyzed different metrics. Type II diabetes is also linked to triggering several other conditions such as heart or kidney diseases. The reasoning behind using again mice fed high-fat diet was that it is known that a high-fat diet is responsible for trigger type II diabetes, but also a poor glucose approach. The three analyzed metrics were GTT, IIT, and PTT, which are all tolerance tests to glucose, insulin, and pyruvate. In this paper, there are explained each one of these tests, such as how GTT is used in order to see how fast one can decompose a glucose quantity out of one's blood, and PTT is used to measure one's ability to produce glucose in a certain amount of time. The figures presented in this section emphasize how for both the GTT and the ITT test mice on a high-fat diet who were administrated gene therapy with AAV:FGF21 either individually or in a combination with the other two managed to have almost the same results as the mice who were fed a normal diet. The way these mice were tested is the following: after fasting for 8 hours (for the GTT) and 6 hours (for the ITT), they've been administrated with a certain amount of glucose and they were tested right after administration, but also a few

more times, the last test being after 2 hours since the administration. Another interesting result that they've noticed is the glucose level right before giving them the 50mg of glucose and making the GTT test was almost the same for the normal-diet mice and the mice who were injected with either individually or combination AAV:FGF21 gene therapy. By the end of this experiment, it was concluded that all three genes may contribute and diminish one's symptoms of type II diabetes, making almost all of its tests similar to a mouse who was not on a high-fat diet.

The third approach medical condition refers to kidney failure and renal fibrosis. Most of the patients having one similar condition, usually end up dying of a cardiac event. For this condition, the two most important genes seemed to be the other two and not the FGF21. In order to simulate this medical condition, there was used a surgical procedure called unilateral ureteral obstruction (UUO), which basically block one of the two ureters partially in order not to make it any more in one's urethra. For this third experiment, there were used mice injected 1 week prior to the surgical intervention. Right before the surgery, on the so-called day 0, these mice were analyzed for fibrosis and remodeling of their kidney, but no effects were shown just because of the previously injected genes. Moving on, the procedure was done and during the time after, the gene therapy showed some effects. Given these results, researchers concluded that this gene therapy may be the one responsible for strengthening one's kidney and they've moved on making a couple of tests. The one results that I'll mention is the MTS scan procedure, which stands for Masson's Trichrome stain and was done 1 week after the UUO surgery. For this test, whole kidneys were harvested and analyzed for seeing what were the effects on each mice category. When looking at these images, the two main things that were analyzed are the renal fibrosis that may be formed, and the way renal medulla was affected. These pictures showed that the largest area of renal fibrosis was found in the control mice, and the mice that were the best protected were the ones who have been previously injected with TF gene therapy. Anyway, all cases of gene therapy showed better results when compared with the control mice, therefor it was shown that these ones have resulted in this case as well.

The fourth and the last experiment that was done is the one for the heart and cardiac failure. In order to simulate this medical condition, it was used another medical procedure

called “Ascending aortic constriction” or AAC which basically had the same purpose as the previous one to obstruct a small part of mice aorta, in order to mimic the heart hypertrophy condition with comes with age-related hypertension. For this last experiment, there were used six-month-old mice, adult age, and they’ve also been injected 1 week prior to the surgery with each gene therapy either individually or in combination. Before the actual procedure, they’ve done some echo-cardiograms for each mice category and there were no significant differences in mice’s heart depending on either they’ve been or not injected with any type of gene therapy. Moving on, to the actual surgical procedure, that was the first time when some effects of gene therapy showed up. During this procedure, the survival rate varied, depending on the gene therapy used. Thus, the highest survival rate was found in mice who were given AAV:sTGFbR2 and AAV:αKlotho, with an 87% survival rate, while the control mice who had no gene therapy done only had a 50% survival rate. For the remaining mice, there were done additional tests such as the fractional shortening metric, the ejection fraction percentage, but also some MTS pictures as in the previous scenario. The first metric, the fractional shortening represents how much a heart can contract during one cycle and this metric should be over 20% normally. Right before the surgery, the fractional shortening was for all categories over 20 percent, but this same metric was measured after 7, 30, and 90 days. During the first check-up, after 7 days, most of the mice from all the categories had their fractional shortening over the 20 percent threshold. Anyway, after 30 and 90 days, it was noticed that more and more mice that were injected with gene therapy started to regain their normal rhythm and their fractional shortening started to be above 20 percent again, while the control mice only decreased more and more in their fractional shortening. Another metric that was examined is the ejection fraction. This one comes in opposition to the fractional shortening and represents how fluid can be evicted during each heart contraction. In one of the figures presented in the paper, there are multiple echo-cardiograms that quantify the number and the frequency of the ejection fraction for the control mice and the gene therapy injected mice. In those echos, one can see how the amplitude and the frequency are much higher in the injected mice than in the control mice. Furthermore, when an MST was done, it was noticed a 61 percent decrease in interstitial fibrosis for the TK gene therapy in comparison to the control mice. In conclusion, for this last experiment, the best results were

shown for the TK and TF gene therapies, while the control mice had very poor handling of this experiment.

5 Conclusion

After running all these four experiments with the previously selected gene therapies, FGF21, αKlotho and sTGFβR2 we can draw the following conclusions: for the obesity model it was shown that all three gene therapies either individually or in a combination for both the adult-aged high-fat diet mice and the old-mice were able to completely reverse or at least remedy the obesity phenotype; for the second model regarding the type II diabetes we notice the same results as in the previous case, any gene combination therapy showed results; for the third model concerning renal failure, the most effective was the FGF21 and sTGFβR2 gene therapy, while for the last model the best results were seen on the mice injected with either FGF21 and sTGFβR2 gene therapy or αKlotho and sTGFβR2 gene therapy. Therefore, it was showed that AAV-based gene therapy with only two genes (FGF21 and sTGFβR2) can successfully treat all four age-related diseases at once.

Thus, this paper demonstrates that treating multiple diseases with one treatment can be possible and opens up new paths of exploration for future medical conditions, that maybe one day transformed in a plausible treatment for humans as well. As they've previously emphasized, the most important aspect is actually to extend as much as we can one's health-span, so that such age-related diseases come at a later age since it seems that they are strongly related to one another.