A Step Towards The Immortality By Increasing Telomere Length By Ipsc's Or Crispr Cas 9

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Abstract: Many people at the time of their death regret of them not doing certain things in their lives. So why not grant them another chance to live their lives upto their expectations. Why do we die? The answer is because our cells stop dividing. Why does cell stop dividing? Because the telomere protecting the DNA at the ends of the chromosomes is reduced to a number after which it doesn't allow any further division, leading to senescence. The telomere can be regenerated by different techniques like iPSC's or CRISPR CAS 9

Index Terms: Telomere, Senescence, iPSC's, CRISPR CAS-9

1 INTRODUCTION

THIS paper focuses on increasing the telomere length of the chromosomes, which is the reason for the cell's senescence. Telomere DNA is related to the DNA-binding protein, and a loop structure which is mediated by the TRF 2 protects the end of the chromosome. Their genomic sequence is TTAGGG which get triggers in the tumor cells these cells uses alternate pathway to maintain the length of the telomere and simultaneously does the division. It was suggested that recombination has something to do with the telomere maintenance. When you see wrinkles they are dead cells of the skin and thus, telomere is also used in the beauty creams that we use nowadays. These telomeres can be considered as shoe aglets that keeps the DNA intact, in a similar way the aglets keep the lace intact. The DNA sequence of the telomere is TTAGGG. So it's recognition and editing is much easier with its sequence known. And moreover these are the repetitive sequence. Telomere plays a vital role at genomic level by protecting the DNA from nucleolytic degradation and unnecessary repairing of the DNA. Telomere length can therefore be considered as a biological clock of one's life.

2 NOVELTY

The idea has not yet been implemented in laboratories still trials are going on but still the lengthening of the telomere has not yet been achieved. But there are many hypotheses. Rather than rebuilding the telomere, another method can be used to hieve telomere lengthening by inducing pluripotency into cells. The basic idea is to achieve a stage between the tumor cells and the normal stem cells. The idea is to induce a tumoric stage into the cells so that they can opt for the alternate pathways and do not further degrade the telomere and cell proliferate but the proliferation should be controlled. When an embryo is embedded to the wall of the uterus, the telomere length of the chromosomes is 15,000 bp which after many division is reduced to the 5,000 bp leading to the senescence of the cells. Various factors are used to induce pluripotency into the cells like- Oct4, Sox2, c-Myc or Klf 4. But different expression of these factors lead to different changes into the iPSC's. When a normal stem cell is converted to an iPSC [1] there is an increase of a telomere length of up to 500 bp, in order to carry on with further divisions. And according to the researches done till now, one single division loses up to 200-300 bp of telomere.

CALCULATION

A normal human being from its birth to death utilizes 10,000 bp of each chromosome.
And,
Human beings average life span is 70-80 years.
This means, for one cell telomere used per year is

=> 10,000\80= 125

Approximately 125 telomere used for a single year from a single cell. And if we are able to increase telomere length up to 500 bp, we can give a person to live more. i.e.- about 4 years of extension. (500/125=4) Another approach towards lengthening of the telomere can be CRISPR CAS-9 technology which has not been fully discovered. The CRISPR CAS-9 [2] technology is basically related to the genome based editing, so it may prove to be a very useful tool to regenerate the telomeres. And changing the gene into the human genome is not easy but the recently developed method will help us to edit the genome precisely and accurately the way we want.

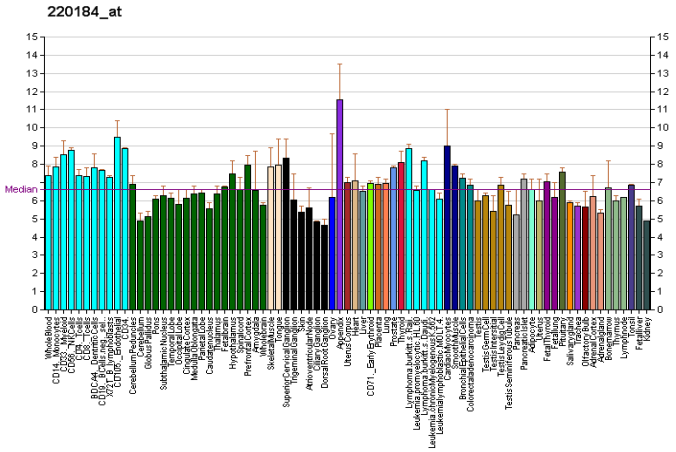
Applicability

Who doesn't want to live? A person dying wishes if he/she could live more, but no one is able to fulfill their wishes. With this approach we may be able to achieve which till date was the tale only imaginable. This approach will help us to cure deadly diseases like cancer. EXPLAINATION: When a person is infected with cancer an organ is badly effected by tumor cells, but not whole of it. What can be done, take out the organ select the non-infected cells and induce pluripotency and different kinds of differentiation can be triggered into the cells like antigen exposure, the genomic sequences or various factors like Nano g. There might be a risk of having an uncontrollable proliferation of cells in that case we can use the STOP gene or the gene that can repress Nanog gene which regulates Oct4. Athletes and weight lifters who are strictly against surgeries can opt for these telomere extended-differentiated cells to counter different kinds of arthritis, chronic body pains letting them to heal instantly and perform good without taking long bed rest that they used to after going through a surgery. Not just this, we may achieve immortality, idea behind this is repetitive inducing of the pluripotency into the cells in order to regenerate the telomere. And if this doesn't work one can go for the CRISPR CAS-9 method. This method is based upon the defense model of bacteria against the viruses' infection by cutting the viral DNA leaving the cut strands behind. The CAS-9 protein will detect the sequence of the telomere and it will help in increasing the telomere length by adding an extra telomere strand. With CRISPR CAS-9 one can even treat the diseases like progeria as the person

suffering from progeria undergoes instantaneous aging process. So the CRISPR CAS-9 can edit and extend the telomere length.

3 DESCRIPTION OF THE PROJECT

The project is based upon the iPSC's helping in increasing the telomere length. But this subject is more than that. Telomeres are the results of the activity of an enzyme known as telomerase. But with the constant cell divisions these telomerase depletes leading to the shortening of the telomere. In this project, we are going to use transforming factors like Nanog, Oct 4, c-Myc and Sox -2. The Oct 4 gene maintains the pluripotent state of the cell, the Sox2 gene is responsible for the cells' self-renewal capacity by hetrodimerizing the Oct 4 gene. c-Myc plays a crucial role by regulating the cellular functions, proliferation and differentiation. These four reprogramming factors induce the pluripotency reactivating the telomerase which leads to the increase in the telomere length. There might be a risk of having an uncontrollable proliferation of cells in that case we can use the STOP gene or the gene that can repress Nanog gene which regulates Oct4. There are possibilities of the various rates of expression on the human body like shown below in the graph about the Nanog gene expression pattern in the various parts of the human body. [3]



The above graph is the Nanog factor expression in the various body parts. The following graph shows the Oct 4 expression in the various body parts which will tell about the difficulty one can face in the delivery of these factors in the body for converting the cells into the iPSC's and then differentiating them by other factors.

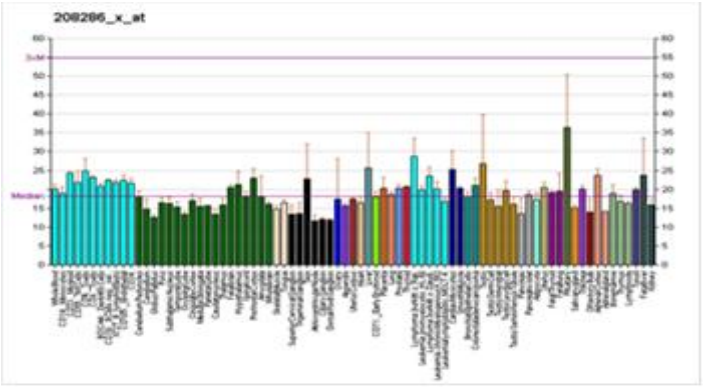


Fig. 2. *Oct 4 expression in various human body parts[4]*

TABLE 1
PRICES FOR ENGINEERED ADENOVIRUSES

Service	Expected titer (PFU/ml)	Price	Turnaround time
Construction of adenoviral vector		Please inquire	5-30 days
Packaging of adenovirus (1 ml): Standard scale	> 10e10	$595	17-25 days
Packaging of adenovirus (10 ml): Large scale	> 10e10	$995	17-25 days
Control GFP adenovirus* (0.2 ml)	> 10e10	Free	

Moreover, the gene therapies in India are priced to be Rs 70,000 /-. Combining the above two costs we can make some approximation of the cost of this total process:

$995* 65= Rs. 64,675 + 70,000

⇨ Total cost = Rs. 1,34,675 (Approx.)

The other method i.e. CRISPR CAS-9 is an effective and promising method. These CRISPR CAS-9 (CAS-9 is a protein complex) can be made to remember the genetic sequence of the telomere as these complex is programmable. So when a telomere (a dna sequence) is broken from somewhere, it will opt two mechanisms. One, repair the broken DNA on its own or else insert the donated dna sequence. In this very crucial process the repetitive DNA sequence of TTAGGG can be inserted into the telomere leading to its extension. CAS 9 molecule after reprogramming can form complimentary DNA strands which will join to the telomere which is cleaved by the CAS 9 molecule. The procedure can be done in the middle length of the telomere and a whole new sequence of the telomere i.e. TTAGGG of 2-10 kb length can be inserted. We had technologies since 1970 but they were insufficient and the CRISPR CAS 9 is considered as the most efficient one due to its precision. We might take the repairing mechanism under consideration since the telomere is also a DNA sequence but it won't happen since the telomere have capping on its surface which protects it and the rest of the DNA from unnecessary repairing mechanism. That is why we will have to insert the artificially synthesized telomere sequence in order to achieve a new lengthened Telomere. This method is much better than the iPSC's because they are tedious and much costlier than the CRISPR CAS 9 method. But there are many challenges that can be faced in the CAS 9 technique because in all the body locations this might not work effectively so the further study has to be done and further modification has to be made. The method might appear cheap but programming the protein and synthesizing it may require a huge amount of money but still this method is more accurate and may revolutionize the world we live in.

IMPACT

The impact of this project on people and environment is discussed below. The iPSC technique, if followed will face a problem of removal of the remaining excess cells in the body which will lead to the cell overloading into the human body. The CRISPR CAS 9 treatment will have to be properly synthesized and have to be delivered to each part of the body.

There will be many challenges faced while performing these procedures on different people because all of the people will react to the treatment differently leading to various outcomes one can think of. This technique if works on the human it might work on the plant leading to their life enhancement and the increase in the productivity. Another problem that can be faced is the TTAGGG is a repetitive sequence, so there might be a possibility of multiple CAS 9 molecules cutting the same telomere at different sites, so what we have to do is we will have to make the CAS 9 molecule chromosome specific in order to cut four telomere lengths of single chromosome for only once. This advancement can bring the revolution in the way we see our world, the way we see our deaths, we will be able to control our senescence. But as everything in the universe has two sides like a coin a good and a bad. It may also have some bad impacts.

REFERENCE

[1] Shinya Yamanakas' Induced pluripotent cells

[2] Jennifer Doudna's, CRISPR CAS 9

[3] GeneAtlasU133A,gcrma.https://en.wikipedia.org/wiki/Homeobox_protein_NANOG

[4] GeneAtlas U133A, gcrma https://en.wikipedia.org/wiki/Oct-4