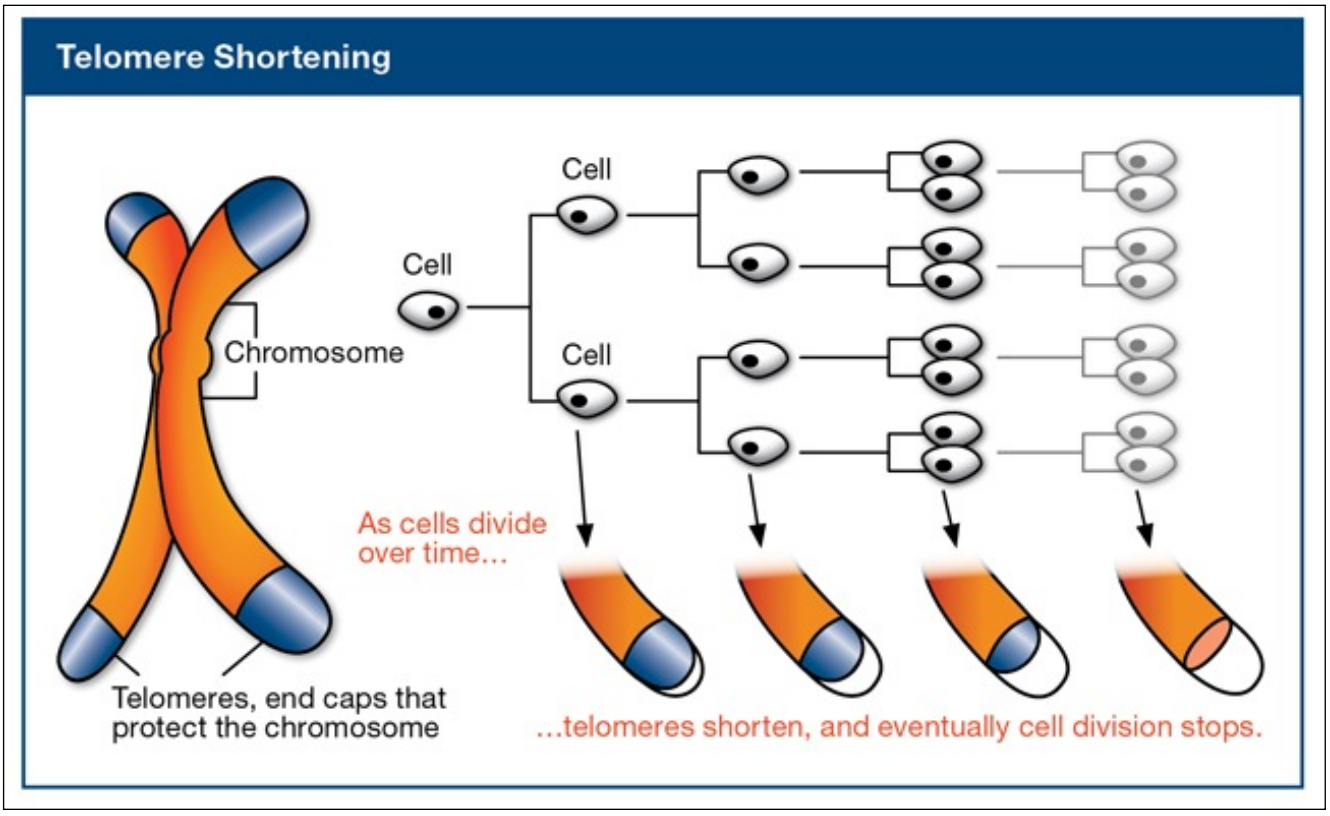
A Novel Bioengineered Adenovirus to Reverse The Effects Of Biological Aging by Replenishing Telomeres



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Abstract

A novel approach can increase the length of human telomeres, thereby reversing the aging process at the cellular level, opening new avenues for treating various debilitating, age-related and genetic diseases. Telomeres are the protective end caps of our chromosomes which shorten with each cell division, finally reaching a critical length, following which the cell stops dividing but the telomeres can be replenished by telomerase activity.

Evaluation of multiple methods to enable telomerase activity at the cellular level, has led me to one viable option - Viral Vectors (Adenovirus or AAV, due to their ability to infect most cell types, and post infection viability of cell being nearly 100%).

Using data from the Human Genome Project, I have been able to identify the DNA Sequence for Telomerase, synthesised by the transcription of a certain gene sequence on chromosome 5.

With current technology, these gene sequences can either be artificially synthesised or extracted from cells. Once this is done, the gene sequence needs to be integrated into the Viral Vector's genome, enabling the virus to infect the cell, expressing telomerase.

The vectors will be genetically engineered to not express viral genes, thus eliciting a weak immune response. Also, the gene sequence responsible for expressing telomerase will remain epichromosomal, thereby being automatically ejected from the cell after a few cell division cycles. This will ensure no accidental inactivation of genes, or activation of oncogenes. The Viral Vector shall be made non-replicative, preventing over-expression of telomerase. These make it an ideal vector to express telomerase.

It is expected to reduce the incidence of cancer, double up as a cancer supressor, Delay and/or ameliorate osteoporosis and arthrosis, Decrease chances of cardiovascular disease, Improve heart, circulatory and lung function, Improve epithelial barrier fitness, Improve glucose tolerance, Decrease insulin resistance, Improve memory function, and Improve neuromuscular coordination, all of which are traits of a youthful organism.

Introduction

Since long, man has been trying to defy aging, a few examples being -

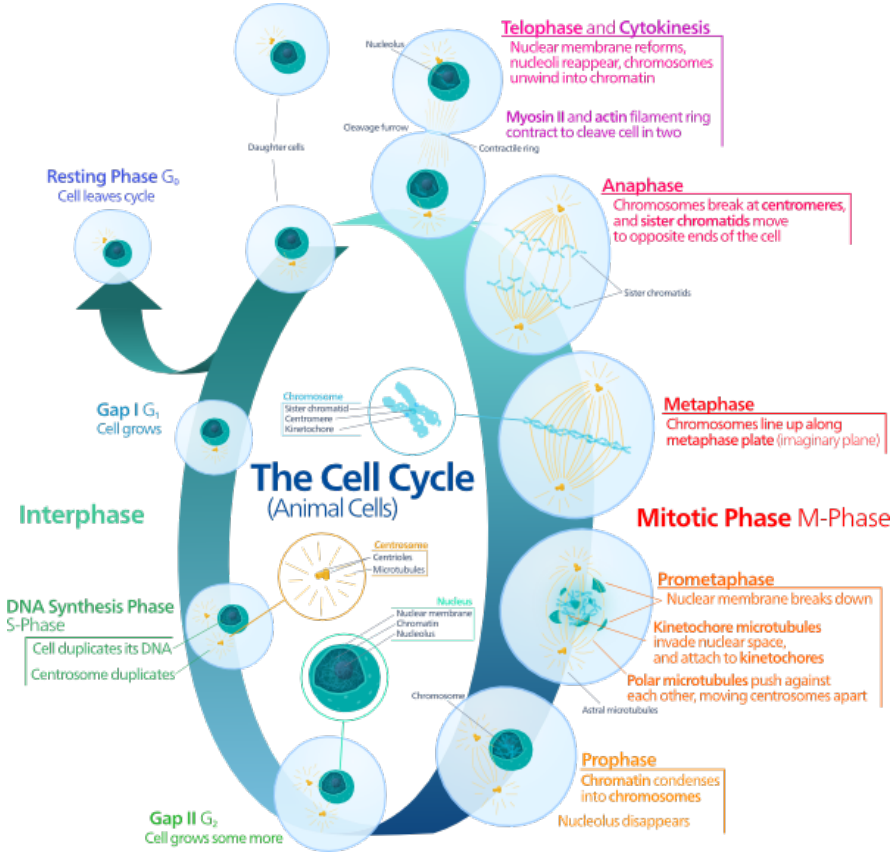
- Use of Frankincense to reverse effects of aging
- Rasayana (Ayurveda)

Lifestyle and dietary interventions can significantly delay aging as can many poor habits accelerate it.

Recent studies have shown that aging is strongly linked to telomere length(Greider et al., 2009) Telomeres are like caps on the end of chromosomes which prevent damage to the chromosomes.

Mitosis

In order to grow and age, our bodies must duplicate cells. This process is called **mitosis**. Mitosis is a process where one "parent" cell divides into two new "daughter" cells. To ensure successful transfer of all genetic information from one generation to the next, each chromosome has a protective cap called a **telomere** located at the end of its arms.



Mitosis comprises of several phases, namely,

- **Interphase** (G1, S and G2 phase)
- Prophase
- Metaphase
- Anaphase
- Telophase

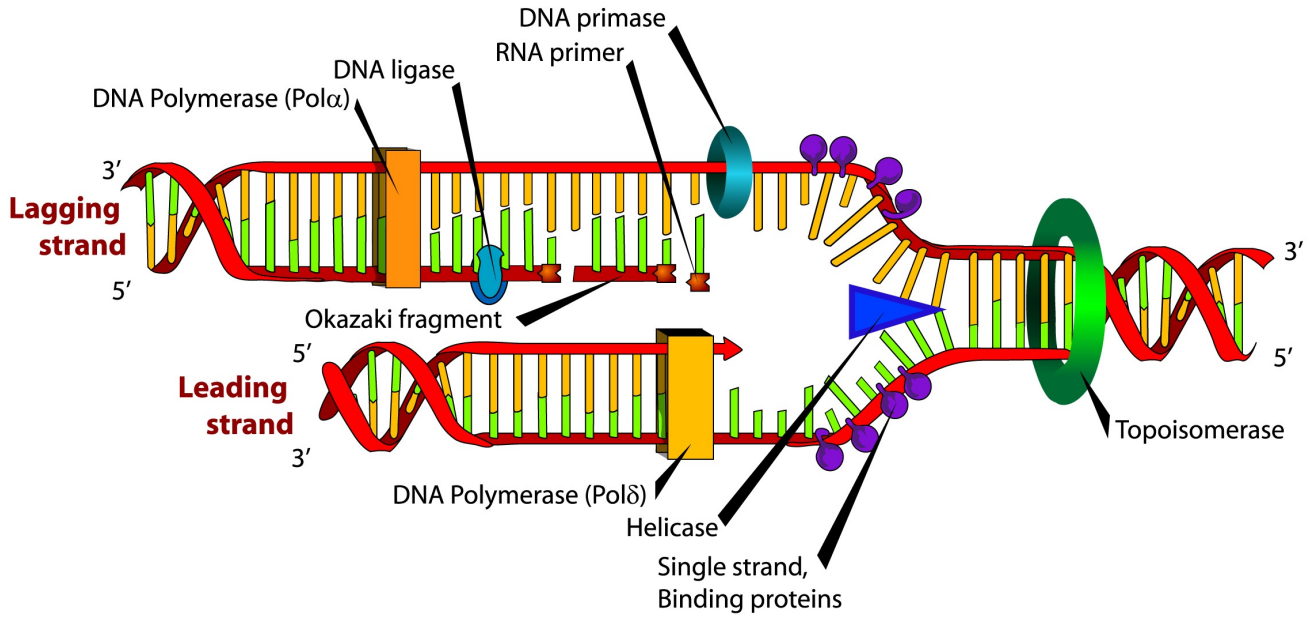
S-Phase

The major event in S-phase is DNA replication. Its goal is to create exactly two identical chromosomes. During replication, helicase unwinds the DNA, and polymerase re-binds complementary nucleotides to the DNA strands.

DNA Replication

DNA replication is the process of producing two identical DNA strands from one original DNA molecule. This process occurs in all living organisms and forms the basis of inheritance. DNA is complementary and each strand of the original DNA molecule serves as template for the production of another strand, a process referred to as semiconservative replication.

In a cell, replication starts from a point called the Origin of Replication. From this point, the replication fork extends in both ways, adding nucleotides to the template strand (Original strand).

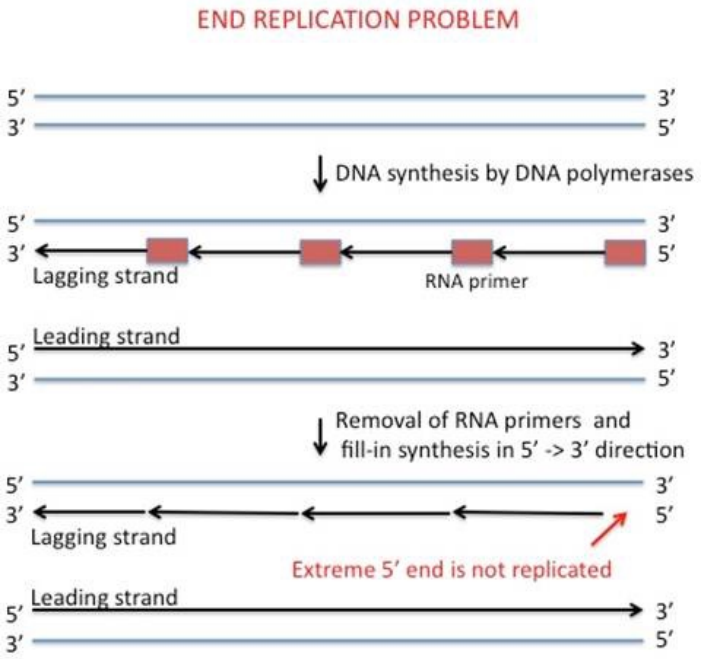


There are multiple enzymes involved in replication -

- Helicase - Unwinds the double helix structure of DNA
- DNA Polymerase - Adds complementary nucleotides
- DNA Gyrase - Relieves strain of unwinding by DNA helicase
- DNA Ligase - Re-joins the strands and joins Okazaki Fragments of the lagging strand.
- Primase - Provides a starting point for DNA polymerase to begin synthesis of the new DNA strand.
- Telomerase - Lengthens telomeric DNA by adding repetitive nucleotide sequences to the ends of **eukaryotic chromosomes**. This allows germ cells and stem cells to avoid the Hayflick limit on cell division.

Now, the DNA Polymerase can only synthesize in the 5′ - 3′ direction, thereby creating a problem while synthesizing in the 3′ - 5′ end. Here, the Primase enzyme comes to use. The Primase adds an RNA Primer to the 3′ - 5′ end, thereby allowing the DNA Polymerase to synthesize the DNA, as now, the primer forms the 5′ end. This strand is called the Lagging Strand.

On the other hand, the other strand is aligned properly. Hence, there is no problem, and the DNA Polymerase synthesizes the complementary strand flawlessly. This strand is called the Leading Strand.



Finally, at the end of the replication process, the Leading strand is complete , whereas, in the Lagging strand, the primer is yet to be removed. Once the primer is removed, there is a gap in the Lagging strand. Due to this, the end of the DNA strand breaks away. i.e. the Telomeres break away.

As this continues, the ability of the cell to reproduce is depleted. Much later, the cell loses its ability to divide.

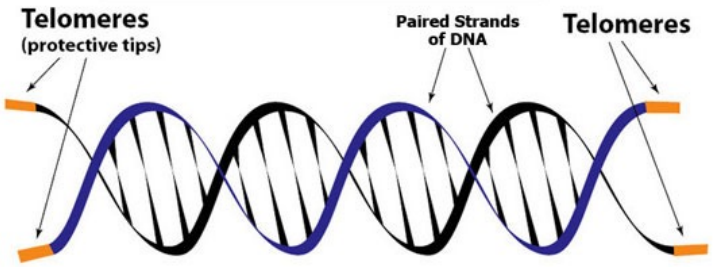
Telomerase replenishes the end of the DNA to prevent loss of vital genetic information.

Telomeres

Somatic cells reach the end of their replicative capacity after a limited number of population doublings (Hayflick Limit). This process has been proposed as a regulatory mechanism which controls the replicative capacity of cells. Telomeres shorten due to incomplete replication at the end of the chromosome.

In the absence of a mechanism to compensate for the end-replication problem, the process of telomere shortening repeats, causing progeny cells to have shorter telomeres than the parent cell, until the cells become senescent and stop dividing. Upon each cell division the chromosomal ends shorten at a rate of 50 –200 bp.

A **telomere** is a region of repetitive nucleotide sequences (5-15 kb) at each end of a chromatid, whose function is to protect the ends of the chromosome from deterioration or fusion with neighboring chromosomes. In humans, the sequence of nucleotides in telomeres is TTAGGG.



Thus, telomeres are disposable buffers at the ends of chromosomes which are truncated during cell division; their presence protects the genes before them on the chromosome from being truncated instead.

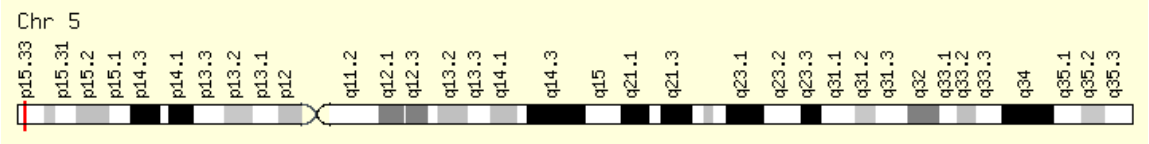
There are approximately 20 kilobases of the telomeres present in human cells.

By replenishing telomeres, we can ensure that the cell doesn't suffer from replicative senescence. It is also worth mentioning that the presence of short telomeres, rather than a decreased average telomere length, forms the ultimate cause of chromosomal instability.

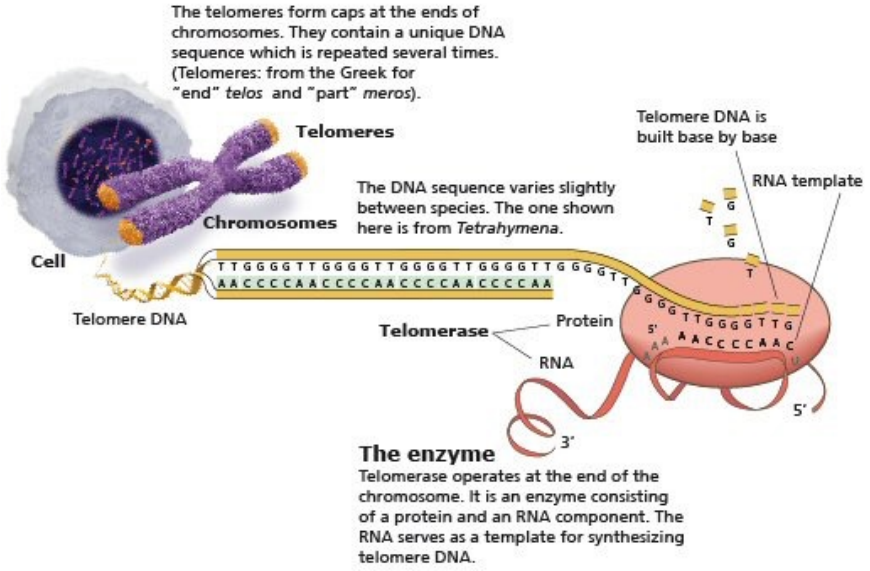
Telomerase

Telomerase is an enzyme which is coded by certain genes present on different chromosomes in the human cell. The expression of telomerase is directly related to which part of the body the cell is a part of. Telomerase is expressed mainly in germ cells, some types of stem cells such as embryonic stem cells, and certain white blood cells. It replenishes the telomeres lost after a cell division cycle.

With the advancements in research and new technologies in molecular biology, we can now identify the genes responsible for telomerase expression and artificially synthesize these genes. These can then be inserted into a cell where these are activated.



Highlighted in red, is the location of the gene responsible for hTERT expression. This is present on chromosome 5.



Introduction to Biotechnology

Biotechnology is the use of living systems and organisms to develop or make products, or "any technological application that uses biological systems, living organisms or derivatives thereof, to make or modify products or processes for specific use". Biotechnology has also enabled emerging therapeutics like gene therapy, which works by activating/inactivating certain genes. *(Wikipedia)*

Gene Therapy

Gene therapy refers to intracellular delivery of genomic materials (transgene) into specific cells to generate a therapeutic effect by correcting an existing abnormality or providing the cells with a new function.

Methods :

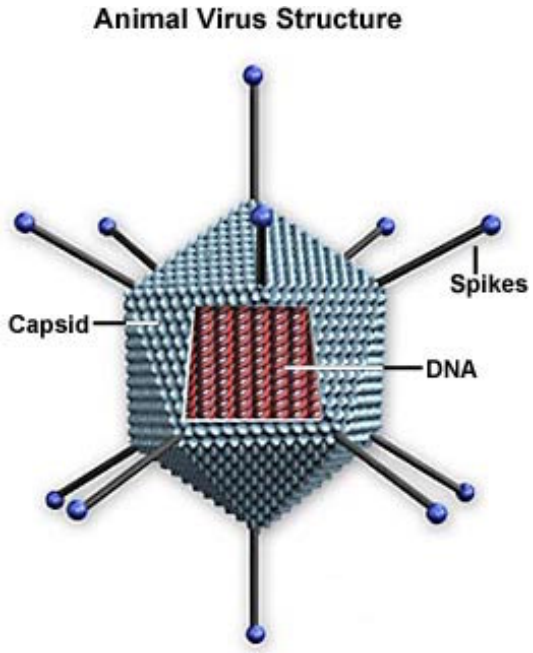
- Non-viral methods such as :
  - electroporation
  - microinjection
  - gene gun
  - impalefection
  - hydrostatic pressure
  - continuous infusion
  - sonication
  - lipofection
  - It can also include the use of polymeric gene carriers.
- Virus mediated gene delivery utilises the ability of a virus to inject its DNA inside a host cell.

There are drawbacks of each method. The non-viral methods generally are less accurate and tougher to perform, as they require precise targeting of the cells to which the genetic material has to be sent.

Viruses are advanced biological machines that efficiently gain access to host cells and exploit the host cell's cellular machinery to facilitate their replication.

Ideal virus-based vectors must harness the viral infection pathway but avoid the subsequent expression of viral genes that leads to replication and toxicity. This can be accomplished by only leaving intact those sequences that are required *in cis* for functions such as packaging the vector genome into the virus capsid.

How do Viruses Work?



- **Capsid** - The capsid is the protein shell that encloses the nucleic acid; with its enclosed nucleic acid, it is called the nucleocapsid. This shell is composed of protein organized in subunits known as capsomers. They are closely associated with the nucleic acid and reflect its configuration, either a rod-shaped helix or a polygon-shaped sphere. The capsid has three functions:

1) it protects the nucleic acid from digestion by enzymes,

2) contains special sites on its surface that allow the virion to attach to a host cell

3) provides proteins that enable the virion to penetrate the host cell membrane and, in some cases, to inject the infectious nucleic acid into the cell's cytoplasm. *(fsu.edu)*

- **Envelope** - Many types of virus have a glycoprotein envelope surrounding the nucleocapsid. The envelope is composed of two lipid layers interspersed with protein molecules and may contain material from the membrane of a host cell as well as that of viral origin. The virus obtains the lipid molecules from the cell membrane during the viral budding process. However, the virus replaces the proteins in the cell membrane with its own proteins, creating a hybrid structure of cell-derived lipids and virus-derived proteins. Many viruses also develop **spikes** made of glycoprotein on their envelopes that help them to attach to specific cell surfaces. *(fsu.edu)*

- **Nucleic Acid** - Just as in cells, the nucleic acid of each virus encodes the genetic information for the synthesis of all proteins. While the double-stranded DNA is responsible for this in prokaryotic and eukaryotic cells, only a few groups of viruses use DNA. Most viruses maintain all their genetic information with the single-stranded RNA. There are two types of RNA-based viruses. In most, the genomic RNA is termed a plus strand because it acts as messenger RNA for direct synthesis (translation) of viral protein. *(fsu.edu)*

Steps of Infection

- Attachment - A virus attaches to a specific receptor site on the host cell membrane through attachment proteins in the capsid or via glycoproteins embedded in the viral envelope. This can be illustrated by thinking of several keys and several locks where each key will fit only one specific lock. *(Wikipedia)*

- Entry - Animal viruses can enter through endocytosis, in which the cell membrane surrounds and engulfs the entire virus. Some enveloped viruses enter the cell when the viral envelope fuses directly with the cell membrane. Once inside the cell, the viral capsid is degraded and the viral nucleic acid is released, which then becomes available for replication and transcription. *(Wikipedia)*

- Replication and Assembly - The replication mechanism depends on the viral genome. DNA viruses usually use host cell proteins and enzymes to make additional DNA that is transcribed to messenger RNA (mRNA), which is then used to direct protein synthesis. RNA viruses usually use the RNA core as a template for synthesis of viral genomic RNA and mRNA. *(Wikipedia)*

- Egress - The last stage of viral replication is the release of the new virions produced in the host organism. They are then able to infect adjacent cells and repeat the replication cycle. Some viruses are released when the host cell dies, while other viruses can leave infected cells by budding through the membrane without directly killing the cell. *(Wikipedia)*

Some viruses generate an immune response. These immune responses can be blocked by removing certain proteins from the surface of the virus, which trigger the response. In this project, we will use a non-replicative virus to prevent over-expression of telomerase.

Types of Vectors

The vector can be of two types :

- Integrative - The integrative vector works on the principle of Cre-Lox Recombination, it is possible to delete the original gene sequence of hTERT, and insert the recombinant sequence. This method causes permanent genetic modification. The action of telomerase can be controlled by the Tetracycline-Controlled Transcriptional Activation method of inducible gene expression, where transcription is reversibly turned on or off in the presence of the antibiotic tetracycline or one of its derivatives.

These vectors may cause apoptosis or cell malfunction, as there are chances of random insertion. This may even lead to cancer.

- Non-Integrative - They contain the genomic sequence of TERT, and don't integrate into the genome.

We choose to use the non-integrative vector.

The method of insertion of external DNA is common to both - The Restriction Enzymes cut the original DNA, and the external DNA is attached there. The action of telomerase can be controlled by the Tetracycline-Controlled Transcriptional Activation method of inducible gene expression where transcription is reversibly turned on or off in the presence of the antibiotic tetracycline or one of its derivatives. The DNA sequence of TERT is generated through many recent methods of artificial synthesis.

Why Non-Integrative?

Using non-integrative viral vectors are advantageous. This is because :

- First, Non-integrative vectors do not cause any permanent genetic modification.
- Second, the vectors incorporate a safety mechanism to avoid over-proliferation of TERT expressing cells. Cells will lose the vector and consequently the telomerase expression if they start proliferating quickly.
- Poor immunogenicity

Examples of suitable non-integrative vectors are - those based on gutless adenoviruses or adeno-associated viruses (AAV).

Suitable Viruses

Table 2. Comparison of Common Gene Transfer Approaches

Features	Adenoviral	AAV	Retroviral	Lentiviral	HSV	Non-viral
Host range	broad	broad	restricted	broad*	restricted	broad
Transducing efficacy	very high	high/moderate	low	low	moderate	very low
Chromosomal integration	no	yes/no	yes	yes	no	no
Duration of expression	weeks-months	long-term	long-term	long-term	days	days
Construction procedure	easy	established	established	difficult	difficult	varied
Transgene size	5 - 36 kb	4 - 5 kb	4 - 5 kb	8 - 9 kb	large	unlimited
Vector yield (titer)	high (>10^{11})	low (<10^{9})	low (<10^{7})	low (<10^{6})	high (10^{10})	high
Host cell proliferation	not required	not required	required	not required	not required	not required
Regulatable expression	available	available	possible	possible	difficult	available
Immune responses	high/low**	low/rare	rare	rare	high	low

Notes *VSV-G pseudotyped HIV vectors
*reduced responses against gutless vectors

- Adenoviruses

There are many advantages in using an adenovirus to introduce genetic material into host cells :

- Represents a homologous system for human genes: adenoviral vectors use a human virus as vector and human cells as host. Therefore, human proteins have identical post-translational modifications as native proteins.
- Has the ability to infect most mammalian cell types
- Accommodates reasonably large transgenes (up to 8 kb)
- Allows high expression of the recombinant protein
- May be grown at high titer
- Is well tolerated, with post-infection viability of the host cells being almost 100%
- Remains epichromosomal, i.e. does not integrate into the host chromosome so does not inactivate genes or activate oncogenes

The E1 gene products, including E1A and E1B, are involved in the replication of the virus. The E2 proteins provide the machinery for viral DNA replication and transcription of late genes. Most of the E3 proteins are involved in modulating the immune response of infected cells. The E4 gene products are involved in the metabolism of virus messenger RNA and provide functions that promote virus DNA replication and shut-off of host protein synthesis.

Though they generate a strong immune response, the responses can be dampened by eliminating surface proteins and certain genes like E1, E2 and E4.

Also, the patient can be subjected to various immunosuppressants over the course of the treatment.

The adenovirus vector is able to deliver genes with 100% efficiency to a wide selection of cell types including dividing or non-dividing cells, or primary cells or cell lines.

All these make AdV's an optimal choice for gene therapy. *(Vector BioLabs)*

- Adeno-Associated Viruses

The unique life cycle of adeno-associated virus (AAV) and its ability to infect both non-dividing and dividing cells with persistent expression have made it an attractive vector. It lacks pathogenicity. Hence, we have considered AAV's as our gene therapy vector.

There are two stages to the AAV life cycle after successful infection, a lytic stage and a lysogenic stage.

In the presence of helper virus (adenovirus or herpesvirus), the lytic stage ensues. During this period, AAV undergoes productive infection characterized by genome replication, viral gene expression, and virion production.

ITR sequences - The ITRs were shown to be required for both integration of the AAV DNA into the host cell genome and rescue from it, as well as for efficient encapsidation of the AAV DNA combined with generation of a fully assembled, deoxyribonuclease-resistant AAV particles.

With regard to gene therapy, ITRs seem to be the only sequences required *in cis* next to the therapeutic gene.

Rep Genes - Rep78 and Rep68, are essential for targeted integration, and viral replication.

By removing the Rep Genes, and the IEE (Integration Efficiency Element, vital for integration) from the viral genome, the virus is rendered non-replicative and non-integrative. This is exactly what we need.

AAV genomes rarely integrate into the host genome, but instead, persist as free circular forms. (Clark et al., 2005) In contrast to the wild-type AAV genomes, recombinant AAV vector genomes do not integrate site specifically into chromosome 19 in human cells in vitro and have been shown to remain episomal in animal models in vivo.

One of the most attractive features of current AAV vectors is the continued expression of the transgene for prolonged periods of time. This is in spite of the extrachromosomal location of the vector. Additionally, the rarity of integration reduces the likelihood of insertional mutagenesis.

Preparation of the Vector

The entire adenoviral genome, with exception of the essential cis elements (5′ and 3′ ITRs and packaging signal) can be removed to generate recombinant gutted (or gutless) adenoviral vectors. These vectors are considered the safest adenoviral vectors due to their lack of viral proteins, and they elicit a very limited innate immune response after administration. They also have a large capacity for exogenous DNA, being able to package up to 36 kb of transgene. Cationic liposomes (Yoshida et al.,2000) can greatly increase the transduction efficiency of these viruses.

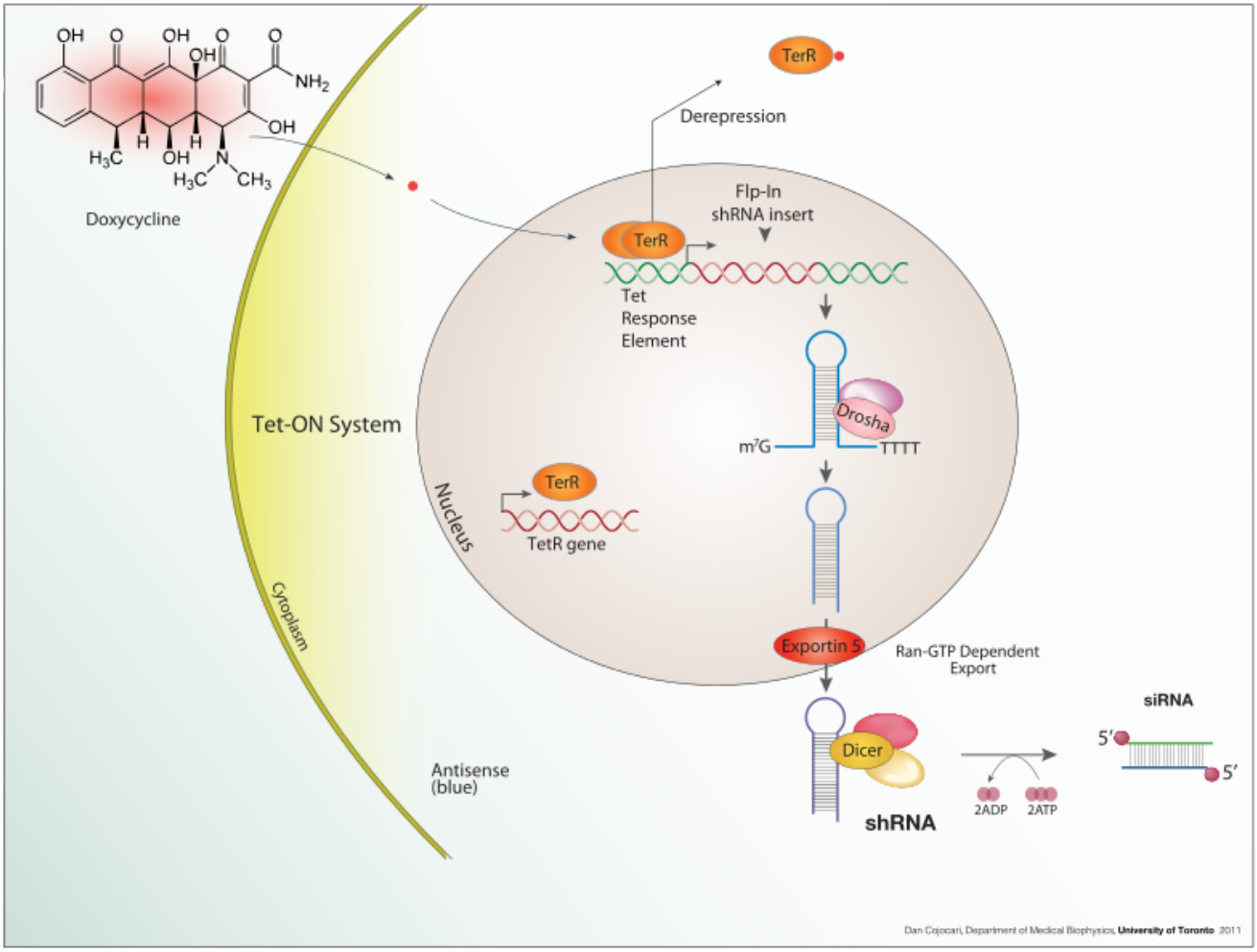
However, their production is a tricky process, as it is known that they can only be propagated in the presence of a helper adenovirus. The major issue in production of gutted adenoviruses is the removal of the helper virus, often a first generation viral vector. Due to the difference in the length of the viral genome between the vector and the helper, the viruses can be separated on a CsCl density gradient by ultra-centrifugation.

The small size of the AAV genome and concerns about potential effects of Rep on the expression of cellular genes led to the construction of AAV vectors that do not encode Rep and that lack the *cis*-active IEE, which is required for frequent site-specific integration. The ITRs are kept because they are the *cis* signals required for packaging. Thus, current recombinant AAV (rAAV) vectors persist primarily as extrachromosomal elements.

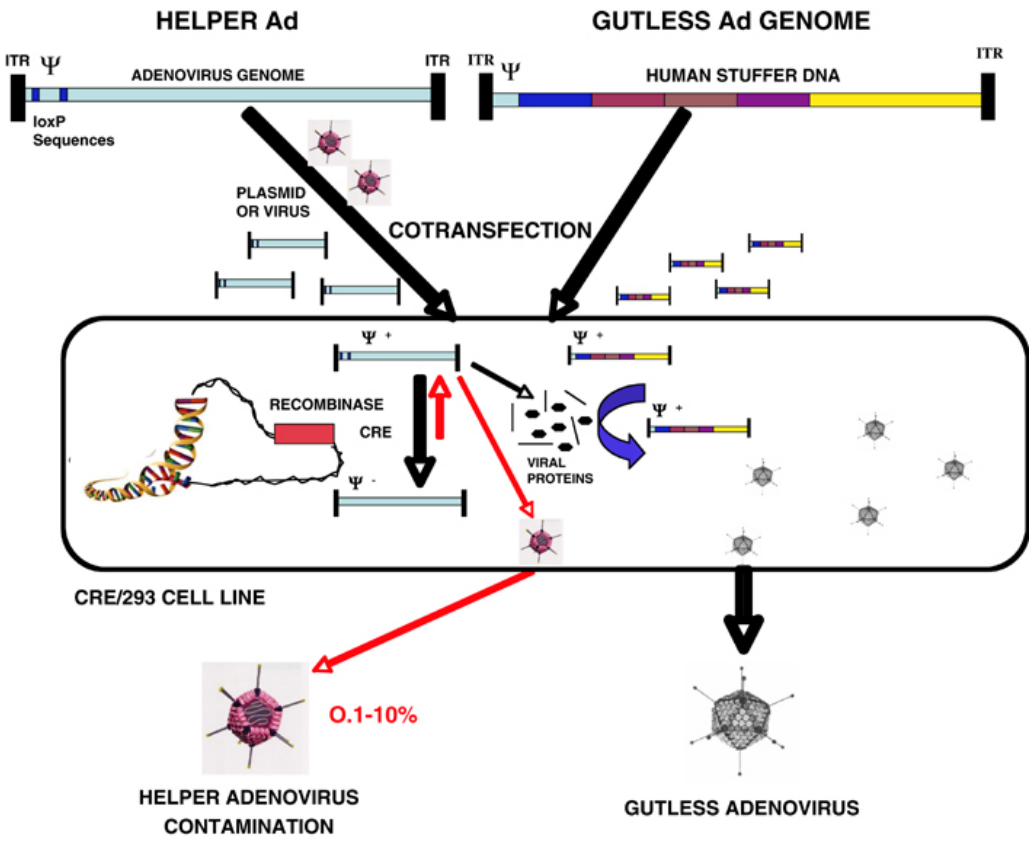
First, the gene coding for the protein is identified. Then, the gene is artificially prepared through Gene Synthesis. Then, the ITR's are attached to both the ends of the human gene. This is then inserted into the virus, and the original viral genome is replaced. These are gutless viruses. They depend on a helper virus to replicate. Since a non-replicative virus is required for the therapy, after the required titer is produced, the helper viruses are eliminated through gradient purification.

The final challenge is to enable transcription of the inserted DNA. This is achieved by the use of a suitable promoter(EF1A), activator(Tetracycline-Controlled transcriptional activation), and start (AUG/CUG) and stop (TAG/TAA/TGA) codons.

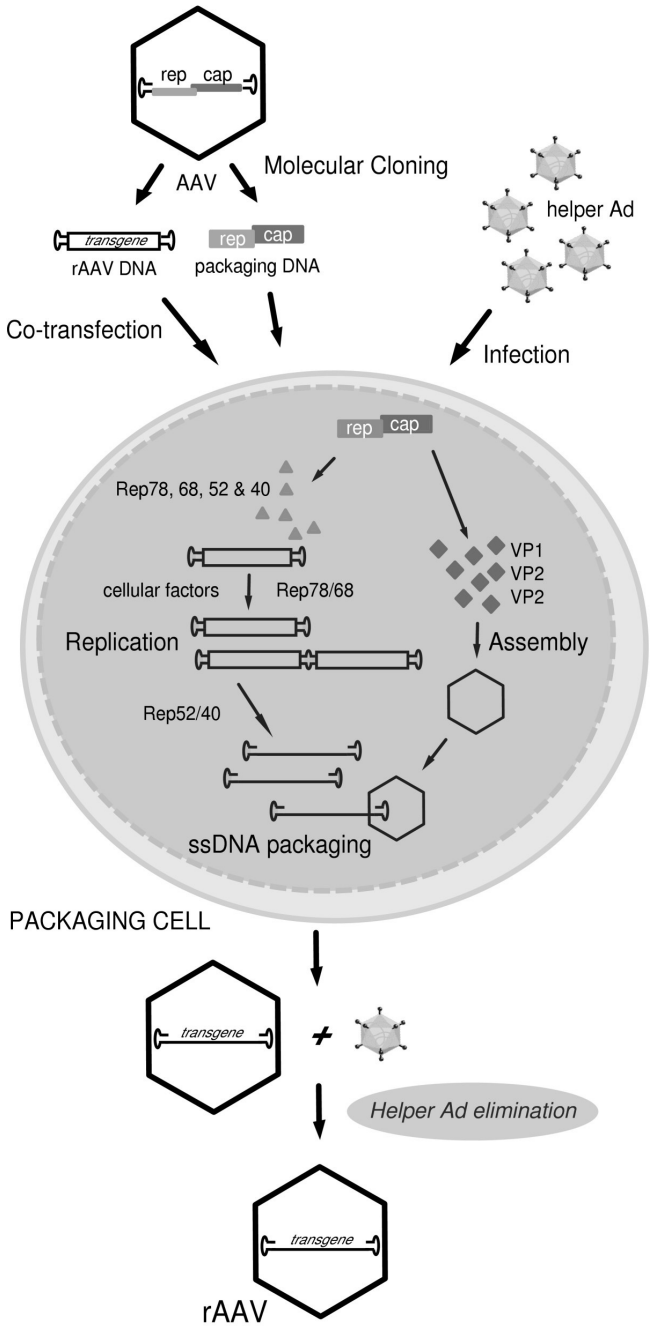
Tetracycline Controlled Transcriptional Activation



After administering the virus, to enable action of TERT, tetracycline or its derivatives like doxycycline can be used to activate transcription, if Tetracycline-Controlled transcriptional activation is used.

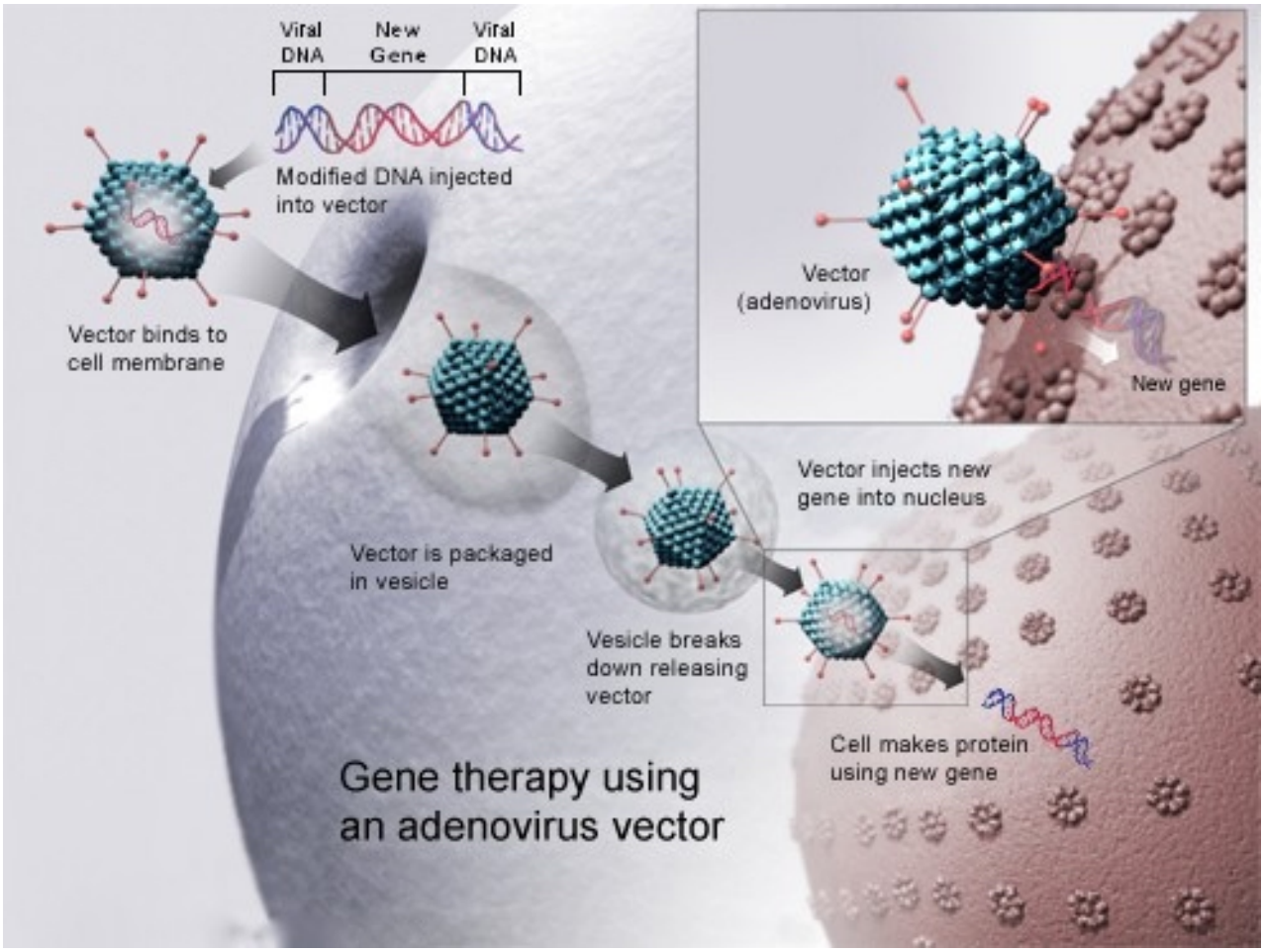


Adenovirus Production



rAAV Production

Working of the Vector



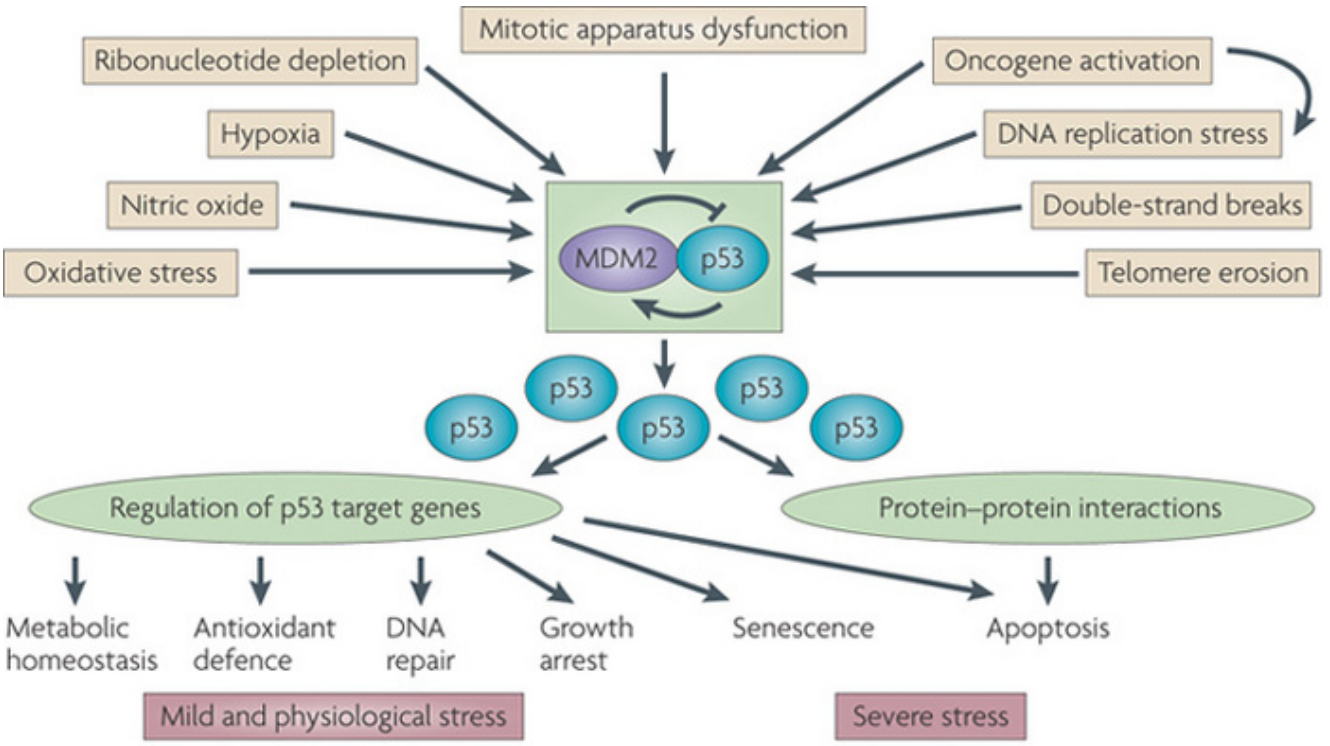
Cancer

- Introduction

Cancer is one of the deadliest diseases in humans, caused by abnormal growth of cells. Most cancers either deactivate the p53 pathway or affect its function in some or the other way. Also, eliminating pRB function seems to be a general requirement of all cancers.

Loss of function of the p53 tumor suppressor gene occurs in more than 50% of all types of human cancers, but mutations in other genes that affect p53 function occur in many, if not all, tumors that retain a normal *p53* gene. Among these are mutations affecting *Hdm2*, *ARF*, and a series of transcription factors that control *ARF* and *p53* gene expression. Therefore, methods targeting p53 are most likely to work.

Also, eliminating RB protein function appears to be a general requirement of all cancers, regardless of disease site. Therefore methods that work on the functional activity of RB are bound to work on and eliminate all cancers.



Nature Reviews | Cancer

- Method 1

The adenoviruses can have a 24-bp deletion in the pRB Binding Domain in E1A. This will prevent them from inactivating Retinoblastoma protein.

Retinoblastoma prevents excessive cell growth by inhibiting cell cycle progression until a cell is ready to divide. In cancers, Retinoblastoma is inactivated, thereby allowing the cell to continuously divide.

If the adenovirus is lacking the Retinoblastoma inactivator, it will be unable to begin S-Phase in the cell, thereby preventing it from acting on the cell.

Rb inactivates E2F. E2F binds and regulates p14ARF, which in turn stabilizes p53.

Therefore, in cancer cells, Rb is inactive and therefore E2F is active. This induces S-Phase. p14ARF expression is induced as cells progress from G0 into S phase. This binds and inactivates MDM2, which allows p53 to accumulate. This may cause the cell to stop replication, and eject the virus, but as this virus has a mutation only in the E1A region, the E1B region acts against this and binds to and inactivates p53.

p53 accumulation directly induces Bax transcription. This induces apoptosis. E1B-19k sequesters Bax and Bak, thereby preventing apoptosis. In most cancers, due to inactivation of p53, the cell doesn't undergo apoptosis.

In case it is active, the following takes place.

E1B-55kDa works by binding a repression domain to p53, converting it from an activator to a repressor of p53-activated genes. This stabilizes p53 and causes a large increase in p53 concentration. Additionally, p53 bound to E1B-55k has an affinity for its binding site that is ten times higher than free p53. Presumably, this increased affinity and concentration of p53 turns the p53-E1B-55k complex into a powerful repressor.

Therefore, these viruses take advantage of the inactivated Retinoblastoma protein. They selectively replicate in these cells, as they can’t inactivate Retinoblastoma.

This mutation also prevents them from replicating, and thereby lysing normal cells, with a functional Rb pathway.

In case the cells have a functional p53 pathway, E1B-55k represses the function of p53, and E1B-19k represses apoptosis caused by accumulation of p53, thus, allowing the virus to replicate within the cell, and lyse the cell once it completes replication.

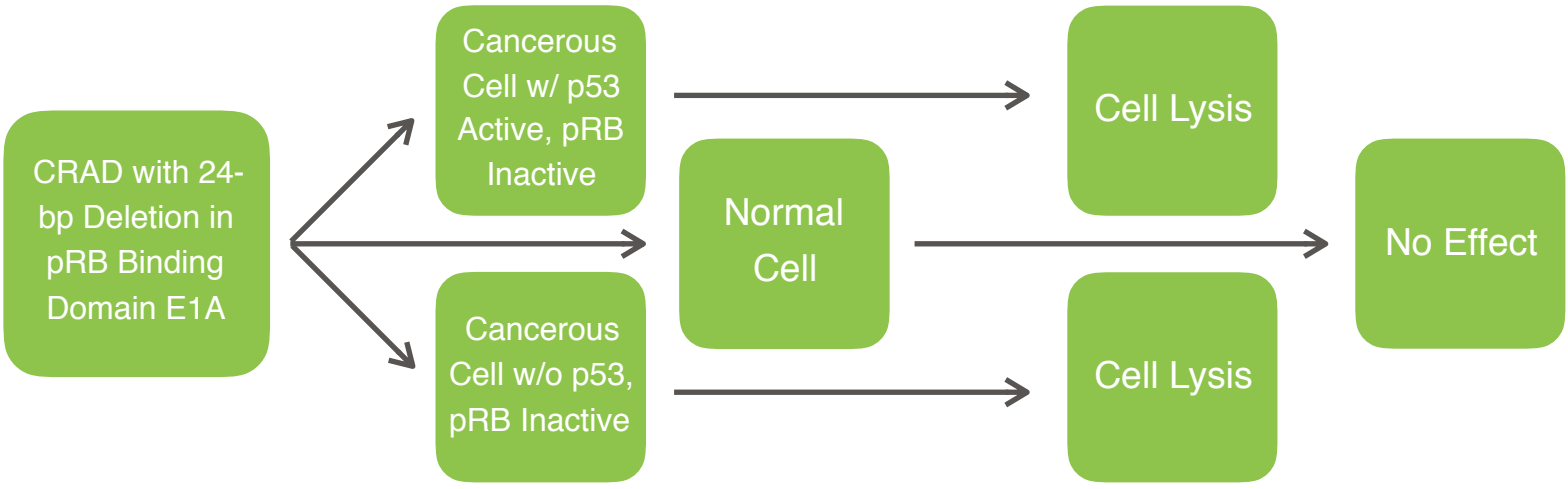
This can therefore theoretically replicate in all the cancerous cells and lyse them.

Retinoblastoma Protein therefore is an important protein in tumorigenesis and sustenance of a tumor. Therefore, it has to be inactivated or disabled in all tumors. We therefore take advantage of this inactivity, and use this against the cancerous cells.

This is therefore a CRAD (Conditionally Replicating Adenovirus).

Since adenoviruses are highly immunogenic, the patient must be kept under immunosuppressants over the course of the therapy, and must be placed in an extremely sterile environment, as any infection may potentially cause death. After the cancer subsides, the patient may be slowly let off the immunosuppressant. Development of a self-subsiding fever may be expected, as few remnants of the virus may still remain in the body.

This therefore kills out the cancerous cells, accomplishing the aim of the invention.



- Method 2

E1B-55kDa works by binding a repression domain to p53, converting it from an activator to a repressor of p53-activated genes. This stabilizes p53 and causes a large increase in p53 concentration. Additionally, p53 bound to E1B-55k has an affinity for its binding site that is ten times higher than free p53. Presumably, this increased affinity and concentration of p53 turns the p53-E1B-55k complex into a powerful repressor.

Removal of E1B-55kDa region of DNA, along with E4orf6 allows the virus to replicate only in cells that have an impaired p53 function. If the virus affects normal cells, any action by it to infect the cell will be in vain due to p53 blocking the replication.

Specific removal of E1B-55kDa along with E4orf6 is required, as research has shown that, in the absence of the full sequence of E1B, the presence of E1A induces programmed cell death or apoptosis in otherwise healthy cells. Induction of apoptosis appears to require exactly the same regions of E1A necessary for induction of DNA synthesis, suggesting that the same signals produced by E1A that induce cell division can trigger suicide. This undesirable side effect would hamper efficient virus production.

My inference from the understanding of the action of E1B is that, when E1A is activated without E1B present, Rb is inactivated and therefore E2F is activated. This induces S-Phase. p14ARF expression is induced as cells progress from G0 into S phase. This binds and inactivates MDM2, which allows p53 to accumulate. p53 accumulation directly induces Bax transcription. This induces apoptosis. But the virus appears to have compensated by evolving the E1B-19kDa protein, which can inhibit apoptosis. E1B-19k sequesters Bax and Bak, thereby preventing apoptosis.

Ad E1A protein can efficiently induce DNA degradation and E1A-induced DNA degradation is significantly inhibited by E1B-19kDa.

Since this therapy is being used to eliminate cancer cells, for safety, E1B-19 kDa protein is required to prevent apoptosis in healthy cells.

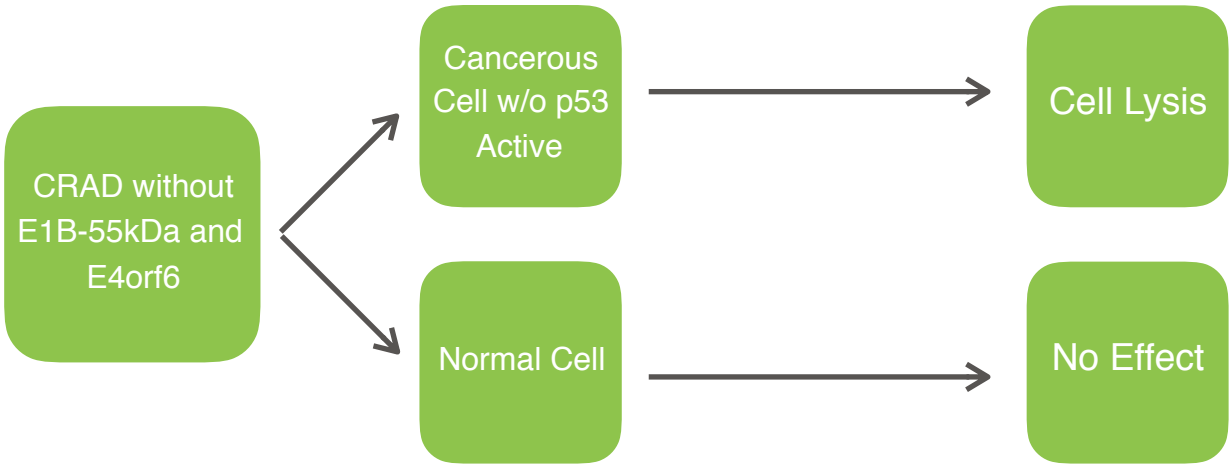
In cancerous cells, p53 function is impaired, and therefore even without E1B-55kDa and E4orf6, the virus is able to replicate. This replication mechanism causes cell death.

The virus will also replicate in cells which have high amounts of MDM2 being expressed, or cells with low amount of p14ARF, or high amounts of E4orf6. Thus eliminating cells prone to cancer.

This too is a CRAD (Conditionally Replicating Adenovirus).

Since adenoviruses are highly immunogenic, the patient must be kept under immunosuppressants over the course of the therapy, and must be placed in an extremely sterile environment, as any infection may potentially cause death. After the cancer subsides, the patient may be slowly let off the immunosuppressant. Development of a self-subsiding fever may be expected, as few remnants of the virus may still remain in the body.

This therefore kills out the cancerous cells, and also cells prone to cancer, accomplishing the aim of the invention.



- Method 3

In addition to **Method 2**, the 24-bp deletion in the pRB Binding Domain in E1A can be introduced.

Such double-mutant adenovirus may further restrict adenoviral replication to a hyperproliferative cell. i.e. Cancer cells.

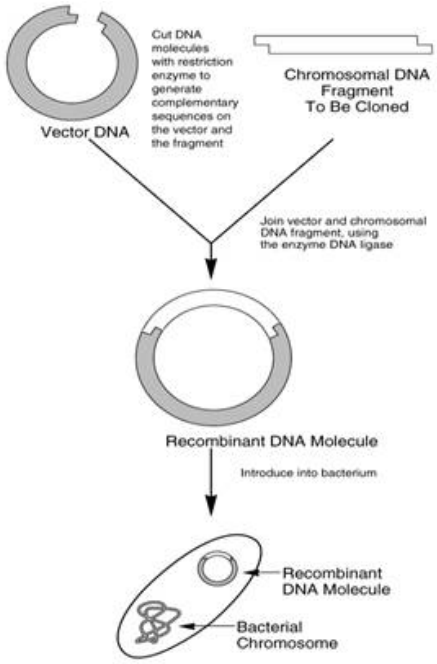
Summary

- Aim

Express Telomerase in Somatic Cells, thereby reversing the effect of ageing on DNA, thus preventing the onset of various age-related disorders, and reducing the incidence of the life threatening disease - Cancer.

- Experiment Design

Restriction sites in Viral Genome and hTERT are identified, Restriction enzyme selected to splice DNA at required site. Specified viral genome removed. hTERT Isolated. Both purified and amplified with PCR (Polymerase Chain Reaction). Overhangs generated. Gene-of -Interest attached to viral genome with ligase. Cloning Complete. Its use in humans is a broad goal, but as of now, initial experiments can be tried on mice.



Due to the difference in the length of the viral genome between the vector and the helper, the viruses can be separated on a CsCl density gradient by ultra-centrifugation.

A control group is injected with the empty virus. Their lifespan is observed.

The experiment group is injected with recombinant virus. Lifespan is observed.

A control group is injected with the empty virus, and kept under high-fat diet. They develop Diabetes Type-II. Their lifespan, and disease progression is observed via GTT (Glucose Tolerance Test).

The experiment group is injected with the recombinant virus, and kept under high-fat diet. They develop Diabetes Type-II. Their lifespan, and disease progression is observed via GTT (Glucose Tolerance Test).

Experiment group should show greater mean lifespan than control group in both experiment designs to validate the theory.

CRADs must show cell lysis in cancerous cells to validate the theory.

- Method

A genetically engineered non-replicative and non-integrative Viral Vector containing the gene sequence of telomerase is introduced into the human subject. By being non-replicative it prevents over-expression of telomerase, while the non-integrative nature ensures no accidental inactivation of genes, activation of oncogenes, and/or prolonged expression as the genes are automatically ejected after a few cell division cycles. Also, hTERT has a half life of ~24h, which is adequate and also complements the needed short-term expression of telomerase.

Two vectors suitable for this project are - Adenoviruses and Adeno-Associated Viruses (AAV9). It is mainly because of their ability to infect most cell types, and them rendering the cells viable post-infection. (AAV 9 targets - Heart, Liver, Brain, Kidney, Muscle, Eye and Pancreatic Cells)Telomerase, responsible for replenishing telomeres is thus expressed.

The subject is treated initially (either oral, intramuscular or nasal), and is then treated again once TERT expression levels decrease by about 50% of those attained immediately following treatment. Treatment may be repeated with the same or alternative vector to maintain the reduction in age-related disorders if necessary, for example annually, or once every 5 years or once a decade.

When administering a second or subsequent dose, it may be necessary to use a different gene therapy vector, for example when using an AAV-based vector the second and subsequent administrations may be a vector with a capsid derived from a different serotype than that used for the first administration. It is possible that a subject may develop neutralising antibodies to the first gene therapy vector, making it ineffective if administered a second or subsequent time.

- Expected Results

The therapy should have beneficial effects in at least one of the following groups :

- Reducing the incidence of cancer, doubling up as a cancer supressor,
- Delaying and/or ameliorating osteoporosis and arthrosis,
- Decreasing chances of cardiovascular disease,
- Improving heart, circulatory and lung function,
- Improving epithelial barrier fitness,
- Improving glucose tolerance,
- Decreasing insulin resistance,
- Improving memory function, and
- Improving neuromuscular coordination.

- Conclusions

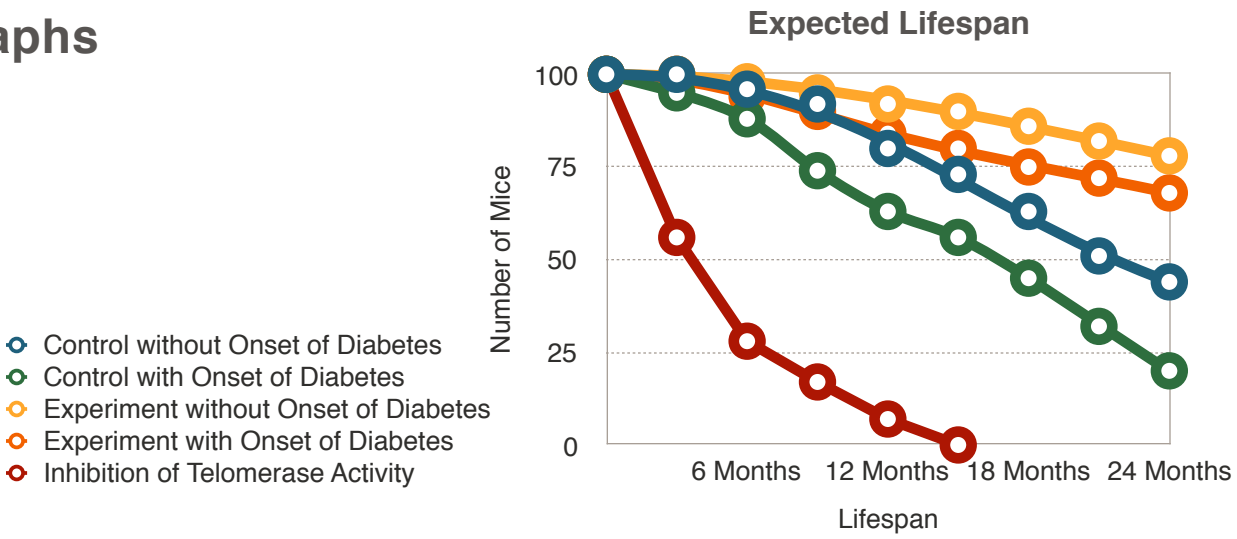
This is a promising field of study. If successful, it can prove to be a boon for mankind. In the future I would certainly be looking forward to approval from Research Institutions to grant me access to perform this research under their guidance.

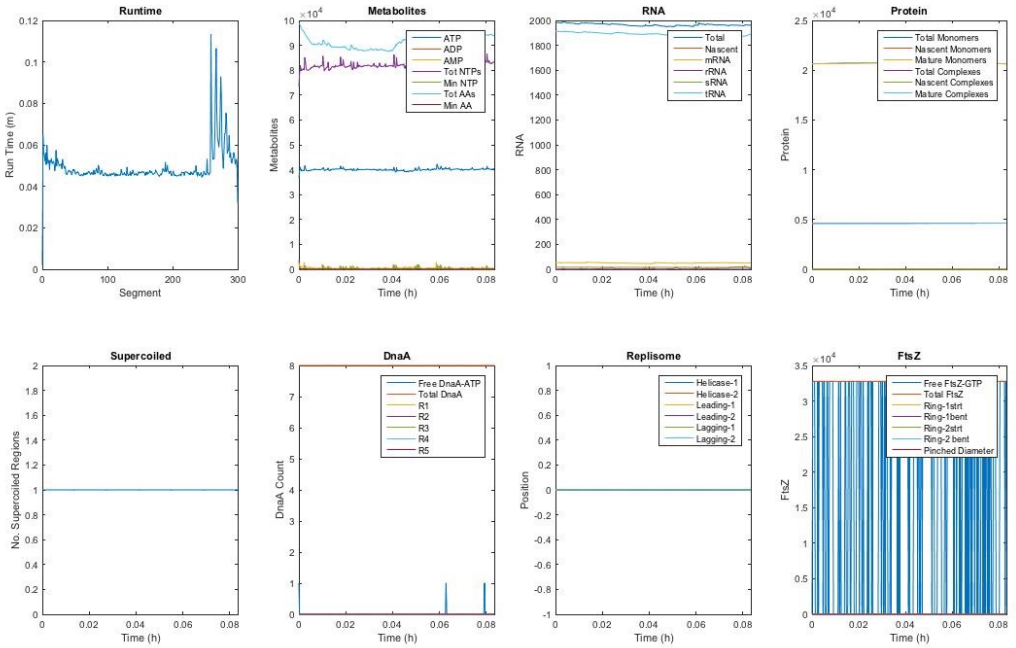
The expression of TERT following gene therapy should persists for a time of several months to several years. Frequent repetition of treatment using the methods and vectors of the invention is therefore not necessary.

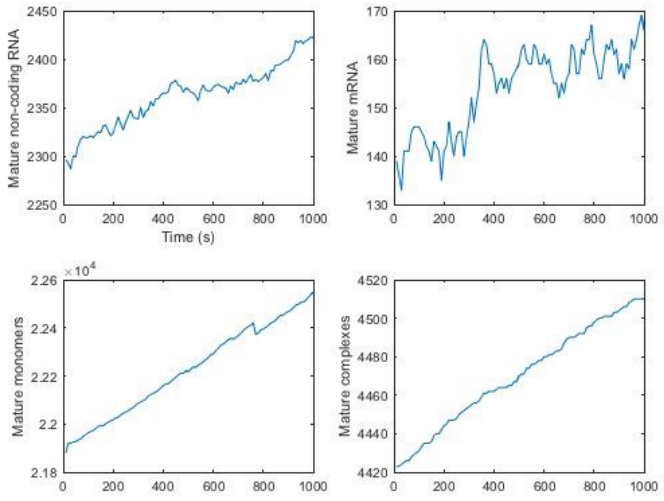
This therapy is mainly directed to adult subjects, displaying secondary sexual characteristics, also be related to mean telomere length.

However, treatment can be administered at other times as well. When a subject is considered to be at particular risk of developing a specific age-related disorder, treatment may be performed before the onset of the age related disorder.

- Graphs


Expected Lifespan
Number of Mice
100
75
50
25
0
6 Months
12 Months
18 Months
24 Months
Lifespan
Control without Onset of Diabetes
Control with Onset of Diabetes
Experiment without Onset of Diabetes
Experiment with Onset of Diabetes
Inhibition of Telomerase Activity


Runtime
Run Time (m)
Segment
Metabolites
ATP
ADP
AMP
Tot NTPs
Min NTP
Tot AAs
Min AA
RNA
Total
Nascent
mRNA
rRNA
sRNA
tRNA
Protein
Total Monomers
Nascent Monomers
Mature Monomers
Total Complexes
Nascent Complexes
Mature Complexes
Supercoiled
No. Supercoiled Regions
DnaA
DnaA Count
Free DnaA-ATP
Total DnaA
R1
R2
R3
R4
R5
Replisome
Position
Helicase-1
Helicase-2
Leading-1
Leading-2
Lagging-1
Lagging-2
FtsZ
Free FtsZ-GTP
Total FtsZ
Ring-1strt
Ring-1bent
Ring-2strt
Ring-2 bent
Pinched Diameter
Time (h)


Mature non-coding RNA
Time (s)
Mature mRNA
Mature monomers
Mature complexes

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