Telomerase and Its Effect on Aging and Cancer

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It has been discovered that the telomeres on the chromosomes of older individuals are shorter than the chromosomes of younger people. This is not due to simple wear and tear on the chromosomes that happens over time, but is actually caused by a flaw in the DNA replication process itself. DNA polymerase is responsible for creating a new strand of DNA by reading the template strand of the existing DNA and sequencing nucleotides to replicate the template strand. DNA, being a double helix, has to unwind and open up in order for DNA polymerase to work. The problem occurs at the end of the DNA strands. DNA polymerase is unable to replicate the last nucleotide sequences at the end due to its size. This causes the new strand of DNA to be shorter than the original. Since telomeres are simply repeats of the sequence TTAGGG and do not code for any proteins, this is not a problem at first. Over time, however, these telomeres keep getting shorter to the point where DNA replication would cause a loss of genetic information. When telomeres get too short, the cell halts any further cell division and is said to become senescent. These shortened telomeres may be the cause of the symptoms of old age. It has also been discovered that the telomeres in cancer cells do not get shorter with each replication, allowing them to proliferate indefinitely rather than dividing themselves to death. Both of these problems can be related to the activity of the enzyme telomerase. Telomerase restores the base pairs lost in the telomeres after DNA replication occurs. It is found in adequate quantities in young cells and cancer cells, but diminishes over time in healthy cells.

In order to determine if telomerase is active in a cell there needs to be a way of measuring its activity quantitatively. Most commonly this has been done by telomerase repeat amplification protocol where a polymerase chain reaction is used to multiply telomerase products that are then measured. This

process can be time consuming and is not the best when measuring telomerase inhibition. Other methods such as fluorescence and electrochemistry are also possible, but may require expensive fluorescent markers or can have low sensitivity. Recently, Wu et al.[1] has discovered a way to use graphene to quantitatively detect telomerase activity with electrochemiluminescence, or ECL. ECL works by having a molecule that will be measured, in this case telomere repeats, bind to an electrode on one end and a $Ru(bpy)_3^{2+}$ cation on the other end. Applying an electric current through the electrode causes $Ru(bpy)_3^{2+}$ to be oxidized to $Ru(bpy)_3^{3+}$. A co-reactant of tripropylamine also present in solution forms a radical due to the electric current. This radical then reduces $Ru(bpy)_3^{3+}$ back to $Ru(bpy)_3^{2+}$ and emits light in the process. The chemiluminescent light emitted is detected by a photomultiplier and is proportional to the concentration of the analyte. What Wu et al. did with graphene was that they attached the graphene, which conducts electricity, to an electrode. The graphene has porphyrins on it to stabilize the graphene so that it does not aggregate in solution and also to add positive charge to the graphene chain without interrupting its conjugated pi bond system. Telomerase substrate oligonucleotides, TS primer, have negatively charged phosphate backbones that are attracted to the graphene because of this positive charge. The nucleotide bases of TS primer are also attracted to the graphene through pi-pi stacking. When this electrode setup is exposed to telomerase, telomerase elongates the TS primer and adds telomere repeats to it. The more telomerase there is, the longer the repeats get. Longer negatively charged DNA repeats will attract more $Ru(bpy)_3^{2+}$, which will then produce more light in the ECL experiment. Figure 3a in Wu et al.'s research paper shows different intensities of light, in ECL counts as units, for different quantities of telomerase labeled "a" through "f". The control of this experiment was label "a" where it only had TS primer, and no telomerase exposure. Label "a" had very low ECL counts compared to the telomerase exposed trials. Trials "b" through "f" were exposed to increasing levels of telomerase, "f" being the highest, and show increasing levels of ECL counts, "f" being the highest again. This figure shows a correlation between telomerase concentration and signal intensity, which means

that ECL can be used as a way to quantify telomerase activity. This method provides advantages of sensitivity and convenience compared to the other methods currently being used and will greatly help in future studies of telomerase activity.

As mentioned earlier, when telomeres get too short they cause cells to stop dividing and become senescent. Since telomerase is less active in somatic cells, telomeres are not being repaired after each cell division in these cells. This gives them a sort of biological clock that ticks with each cell division. After a cell has divided so many times, the telomeres will become too short to divide further. Since at some point human cells can no longer divide to produce new cells, signs of aging start to show. Cancer cells, however, are immortal and can keep dividing indefinitely. This is because telomerase remains active in cancer cells and continuously repairs the telomeres after each cell division. If telomerase could be activated in somatic cells, the cure for old age could potentially be found. Gene therapy would need to be used in order to re-activate telomerase translation. One possible problem with this is for older people with already shortened telomeres. In vertebrates, telomeres are normally protected by a complex of six proteins called shelterin. This complex prevents the fusion and recombination between two different telomeres. Telomere ends of two different chromosomes can sometimes fuse together and cause problems such as genomic instability, cell death, or even cancer. In an experiment by Amiard et al.[2], a similar complex called CST that is found in plants was experimented within the plant Arabidopsis. Its genes were mutated to not produce CST and they examined what happened to the telomeres. Fluorescence spectroscopy was used to measure the lengths of the telomeres by attaching fluorescent markers to them and measuring the relative intensities. When CST was removed from the telomeres, telomerase would lengthen the telomeres, but had to compete with recombination pathways and did not lengthen telomeres all that much. When the recombination pathway was knocked out, however, telomerase was able to lengthen the telomeres much more. This study shows that telomerase can still be used to lengthen already shortened telomeres. This information

is valuable in restoring youth to older cells. By removing the complex that protects telomeres, and inhibiting the other recombination pathways, shortened telomeres can possibly be restored to their original lengths.

There are currently drugs that inhibit telomerase in order to fight cancer. It can be either used alone or in tandem with other cancer treatments. Since telomerase is an enzyme that repairs telomeres and keeps cancer cells immortal, inhibiting telomerase has been proposed as a way to help fight cancer. By taking away the immortality of cancer cells, they will eventually divide themselves into senescence and then die. Their telomeres would eventually get too short to keep dividing. There are many different ways to inhibit telomerase including small molecule inhibiters, G-quadruplex stabilizers, antisense oligonucleotides, and inhibitors of telomerase-associated proteins. In a study by Azhibek et al.[3], modified oligonucleotides were used to intervene in both telomerase assembly and activity. They bought commercially available oligonucleotides and modified them to have either two 3' ends or two 5' ends. They characterized them using MALDI mass spectrometry. Polymerase chain reaction, PCR, was used to quantify the level of telomerase inhibition. Their results showed decreased telomerase activity and assembly both in vivo and in vitro. These modified oligonucleotides could provide for a safe anticancer medication. Laster et al.[4] in their study used a well-known Tetra (4-N-methylpyridyl) porphyrin, or TMPyP4. In this study, they added a palladium atom to the porphyrin and incorporated it into their polylactic-co-glycolic acid, or PLGA polymer rods. These rods containing the porphyrin have shown to decrease lymphoma cell survival in in-vitro studies. They did this by placing the rods in RPMI growth media for the tumor cells, and then placing the tumor cells on the RPMI media. There were two control groups along with this study, one with normal RPMI media, and another with rods that had no porphyrin in the RPMI media. The porphyrin rods group had the least amount of cell growth, followed by the empty rods, and the normal media control group had the largest cell growth. These studies show just two examples of how telomerase inhibition can be used to fight cancer.

One problem with these anticancer drugs, however, is the need to constantly be inhibiting telomerase. If treatment is stopped, then telomerase will reactivate and repair any shortened telomeres. Also, by inhibiting telomerase in the body, cells that constantly need to be dividing like hair and stomach cells will not be able to repair their telomeres. Wai-Yin Sun et al.[5] discovered certain types of gold (III) porphyrin molecules that show anticancer properties that do not inhibit telomerase. They tested their porphyrin's cytotoxicity by placing them in 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, or MTT, assays with different cancer cell types. Living cells reduce MTT into formazan, a purple molecule, which can then be quantified using absorbance spectrophotometry. The control that they used was normal lung fibroblast cells. Their results show that some of the porphyrins they tested were much more cytotoxic to cancer cells than to healthy cells. To determine if the mechanism of these porphyrins was telomerase inhibition, they ran PCR stop assays to examine G-quadruplex stabilization. Small molecules such as cationic porphyrins are known to bind as ligands to the guanine quadruplexes that telomeres sometimes form. This ligand binding helps hold the quadruplex together. G-quadruplexes cause a downregulation of the transcription of c-myc, a gene that has been linked to telomerase. For this experiment, they did PCR on a Pu27 oligomer that forms double stranded products in the absence of G-quadruplex. Polyacrylamide gel electrophoresis is then used to determine if PCR products were formed. Their results showed that four out of the five of their porphyrins that they tested did not inhibit PCR and thus they did not produce G-quadruplex stabilizing agents. This means that they do not kill cancer cells via telomerase inhibition. Another way of protecting the telomerase of healthy cells is to keep the telomerase inhibitors localized to the cancer cells. In the Laster et al. experiment, their drug delivery system that allowed for the continuous release of a cationic porphyrin, the inhibitor, inside tumor cells only. Their drug delivery PLGA rods are inserted into cancer cells directly where they continually release the porphyrin drug into the cells. To measure the rate the rods release the porphyrin in vitro they used UV-Vis spectroscopy and measured the change in absorbance at 415 nm

over time. The best thing about this is the very small amounts of the porphyrin found in the blood plasma after treatment. Calculating the concentration of porphyrin in the blood by using UV-Vis spectroscopy showed high levels of porphyrin initially, but low levels as time went on. These high initial concentrations were attributed to rods that were damaged as they were inserted into the tumor cells. This means that the anti-cancer drug is staying localized to the cancer cells over time.

A lot of research is being done on telomerase inhibitors to treat cancer, but there is not as much research on stimulating telomerase in healthy cells. Increasing telomerase's abundance could potentially increase the human life span, however, it is possible that promoting telomerase activity in a healthy individual could also increase their likelihood of getting cancer. One possible way of promoting longer life while fighting cancer is to increase telomerase activity in the body and then use cancer treatments that do not inhibit telomerase. Another way is to use drug delivery systems to inhibit telomerase only in the cancer cells. Being able to control this enzyme's activity could potentially be both the cure for cancer and old age.

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