Yeshiva University

Journal of Medicine and Dentistry

2023 • Volume 2

Yeshiva University
Journal of Medicine and Dentistry
Volume 2 2023

Mission Statement

The Yeshiva University Journal of Medicine and Dentistry was created for the student body and faculty of Yeshiva University as well as for the broader communities with which the university is associated and for the world at large. It is designed to serve as an outlet for all those interested in publishing work for a public audience. The editorial board consists of undergraduate students from Yeshiva College and Stern College for Women. Writers share analytical essays on new medical developments as well as research they have conducted. This journal aims to foster a greater interest in and appreciation for medical and dental sciences at the university and ultimately aid in Yeshiva University's mission to serve as a "wellspring of wisdom."

BS”D

Letter From the Editors

Dear Reader,

We are thrilled to present the second volume of the Yeshiva University Journal of Medicine and Dentistry.

This volume showcases a wide variety of research papers and reviews written by the Yeshiva University community, including faculty and students from Wilf and Beren campuses. With this being our second year on the Yeshiva University Campus, we have seen tremendous growth, and we are so proud to receive feedback from you, readers and writers. Our journal is being mentioned in classes, professors are encouraging students to publish their work with us, and we are being featured on Yeshiva University platforms as a blueprint for an academically and socially successful club.

As Editors-in-Chief, we have had the privilege of witnessing the remarkable evolution of each paper, from the initial stages of editing to the final product. We are confident that the dedication and rigorous research undertaken by the authors and editors are evident in the exceptional quality of the papers contained within this volume. We are confident that you will find the papers not only informative but also a true representation of the hard work and intellectual rigor that have gone into their creation.

As we look to the future, we remain committed to fostering a collaborative environment that encourages the exchange of knowledge and ideas. We invite you to actively engage with the journal by submitting your own contributions and sharing your feedback. Together, let's shape the future of medical and dental excellence.

Thank you for your continued support.

Sincerely,

Isaac Silverman and Naomi Fried
Editors-in-Chief
Yeshiva University Journal of Medicine and Dentistry 2022-2023

Yeshiva University Journal of Medicine and Dentistry

Research

Three-dimensional Nanoparticle Assembly by a Modulated Laser-induced Microbubble for Fabrication of a Micrometric Pattern

Yosef Weiss

Abstract

This project explores the use of laser-induced microbubbles for both two-dimensional and three-dimensional printing. At its most basic level, this novel printing concept begins with a laser heating up a nanoparticle substrate. The thermal energy input to the system forms a microbubble, and its corresponding convection currents pin nanoparticle deposits at the substrate/bubble interface. Extending this style of printing into three dimensions presents unique issues when exploring two different printing techniques. For the layer-by-layer technique (similar to a conventional 3D printer), wall height irregularities occur due to inconsistent deposition. To solve this problem, the velocity of the stage is programmed to decrease or increase in line with the areas which need more or less deposition. For the vector technique (similar to a printing pen), structures not parallel to gravity lose their stability and consistency. To correct this defect, a double-axis rotating stage was constructed in order to keep the build parallel to gravity to ensure a consistent print for any combination of shapes. So far, this project has successfully evened out layer-by-layer prints and has achieved the rotation required to construct slanted builds.

Introduction

As technology continues to rapidly evolve, the demand for manufacturing precision components has drastically increased. With recent technological developments, society has trended towards smaller devices, which initiated a need for more precise manufacturing techniques. People are accustomed to the concept of three-dimensional printing on the macro scale, but newer technology, including this project, allows for exploration of the micro-scale.

In order to reach this level of precision, the original materials used in printing must consist of nanoparticles or ions. These ultra-fine particles are harvested from the gas products of chemical reactions and are then spun into a dispersion with a different liquid, referred to as a substrate for the means of this project. The method utilizes a unique optical system consisting of a laser to heat the substrate and a microscope to observe the prints. The amplitude of this laser must be "modulated" by rapidly opening and closing the shutter.[7] All of these components combine into one integrated system through which we print on the nanoscale.

Underlying Mechanism: 2D Printing

In the first step of this novel printing method, the nanoparticles/ions are mixed/dissolved into a solution. The underlying mechanism requires focusing a laser beam either into the liquid containing those particles or at the liquid/air interface. Through that laser medium, energy is transferred into the substrate and causes a microbubble to form. Due to this heating, convection currents surround the microbubble and pin deposits at the liquid/bubble interface.[1,2]

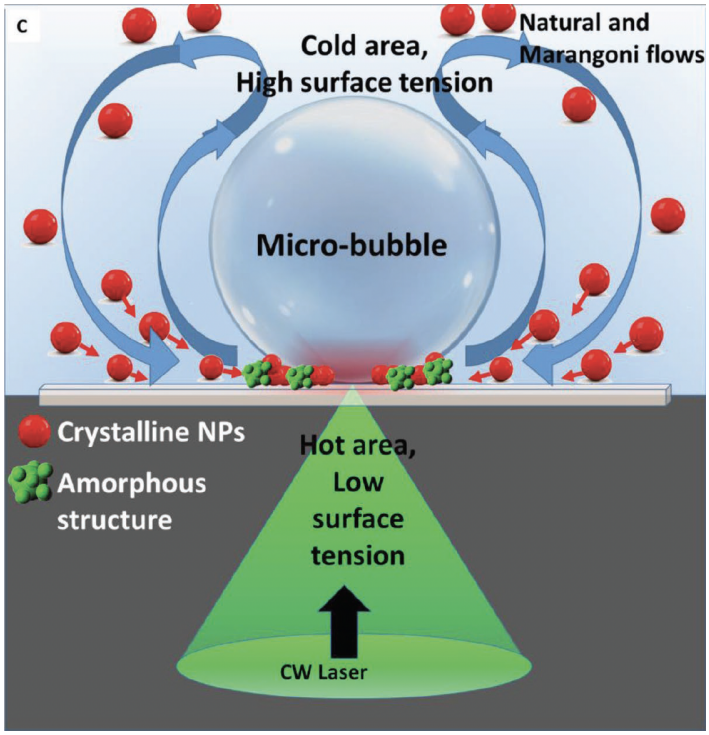

Figure 1. *Convection currents pinning deposits at the junction between microbubble and surface. (Reproduced with permission)*[1]

Over the past nine years, a reliable method of two-dimensional, nanoparticle laser printing has been developed. The overall printing arrangement mainly relies on an optical system consisting of both a modulated continuous-wave laser and an inverted microscope (Figure 2). The laser reflects through the objective lens and onto the sample which is where the bubble forms.[3,4] Without modulation, the microbubble forms, totally collapses, and moves on;[5,6] however, the use of laser modulation doesn't allow the bubble to totally collapse. Rather, the microbubble partially collapses and then advances and reforms, creating a much smoother line. Benefits of this printing format include achieving extremely narrow and precise lines (on the order of nanometers), the ability to print with a wide chemical variety of nanoparticles, and a seamless transition between many different nanoparticle chemistries.

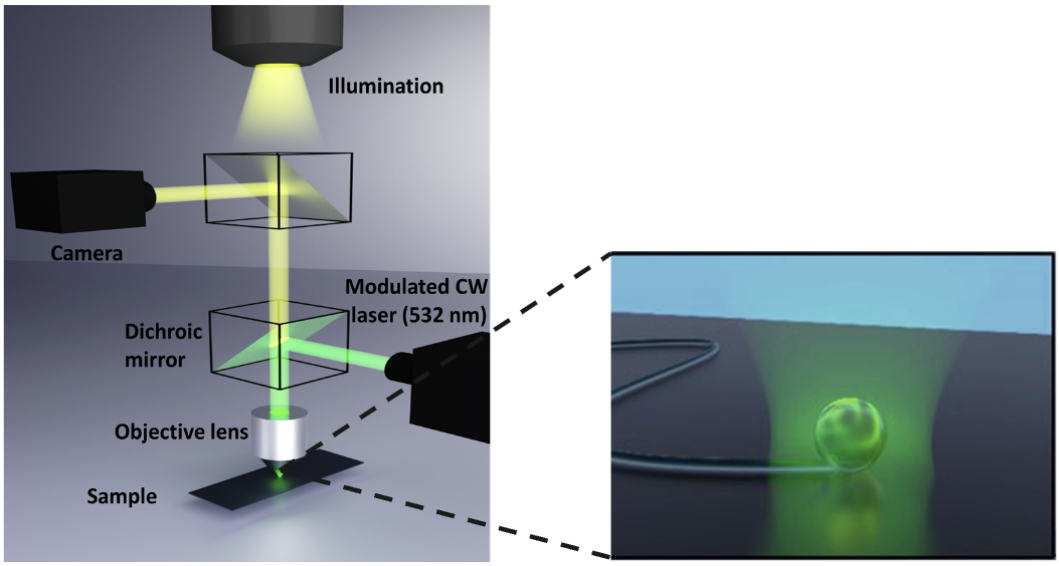

Figure 2. *Optical system consisting of modulated CW laser and optically inverted microscope.*

Extension to 3D Printing: Layer-by-Layer

When extending the project into the third dimension, there are two main approaches: layer-by-layer and vector printing. The layer-by-layer style of printing has a design similar to a traditional three-dimensional printer but employs the laser technique described above. In this method, layers of material are stacked on one another to construct an object. However, when utilizing this printing style, an issue occurs during the layer deposition process: inconsistent material deposition. The origin of this phenomenon is a feedback-enhanced process starting at the very first printed layer. A minuscule sinusoidal pattern of "hills and valleys" of material presents itself, and the areas with slightly higher deposition tend to absorb more light, thus leading to even more deposits at the same location with each additional layer. Essentially, in areas with hills, more deposition occurs than in valleys. As the number of layers increases, the difference between the high points and low points also increases which leads to very obvious irregular wall formations (Figure 3). In fact, in the camera following the laser, one can see the microbubble "jumping" in between peaks as it naturally attracts toward the higher areas.

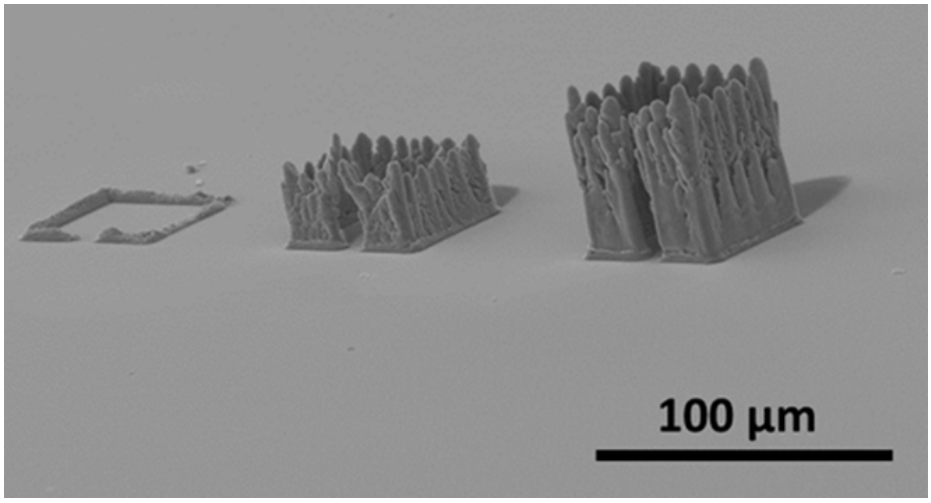

Figure 3. *Stages of wall defect formation.*

The first step in developing a solution to this phenomenon involved identifying the optimal laser parameters for this style of printing, including the intensity of the laser, the frequency of the modulation, the translational velocity of the printing stage, and the duty cycle (percentage of modulation that the laser is on versus off). After extensive testing, two sets of optimized parameters were identified. The first set included a lower duty cycle and intensity combined with a slower-moving stage to allow for more time for the deposition. The second set used a higher duty cycle and intensity but moved at double the speed of the first set. Additionally, an improvement was discovered when the testing lines were printed at a frequency of 100 kHz instead of the previous 1 kHz. To visualize the pattern of deposition in the wall microstructure, a profilometer (optical probe) was used to simultaneously characterize the height and roughness of the lines.

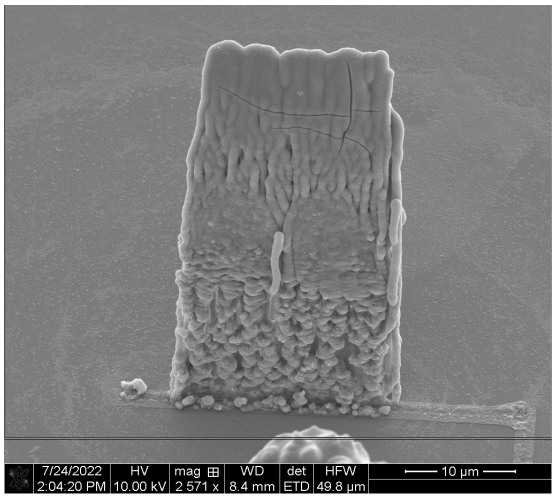

Figure 4. *Scanning Electron Microscope image of set 1.*

It was previously determined that the less the laser is on, the less deposition occurs due to a shorter period of nanoparticle heating.[7] To solve the problem of inconsistent deposition in layer-by-layer printing, a modulating laser was programmed to speed up and slow down depending on the height of the previous deposition at that point. Near the peaks, the stage is programmed to speed up so there is less time for deposition while it will slow down at the valleys. This dynamic control method evens out the height of the layers to ensure a smooth printing wall. Through analyzing multiple wall examples, the timing and frequencies of the pattern of deposition were determined and incorporated into the modulation calculation of the laser to correct (smooth) the deposition pattern, thus making the laser more accurate. By the conclusion of this portion of the project, the walls contained more pillars in the lower section and showed a smoother consistency in the upper section.

Extension to 3D Printing: Vector

The vector method of the three-dimensional printing process directs the laser onto a continuously growing "stack" of material—very similar to the concept of a 3D printing pen (Figure 5). When printing vertically, this method works beautifully; however, an issue arises when the laser deviates from a parallel-to-gravity, pillar-like structure.

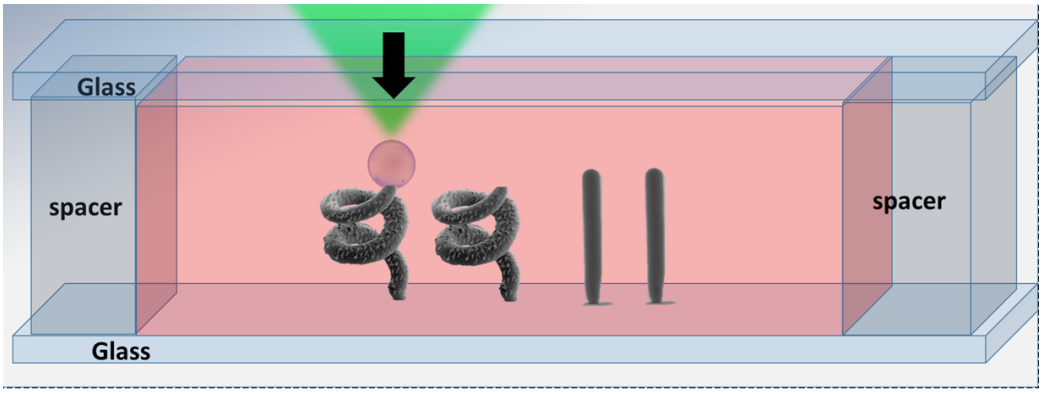

Figure 5. *Vector printing setup.*

In these cases, the structure loses its stability and consistency leading to an undesirable product (Figure 6). This occurs due to a detail within the deposition mechanism of the microbubble. When printing on a slant, the newly forming bubble depositions, no longer sit directly on top of the location of the previous bubble. Deposits tend to accumulate unevenly at the bottom of the microbubble leading to the deformation of the microstructure.

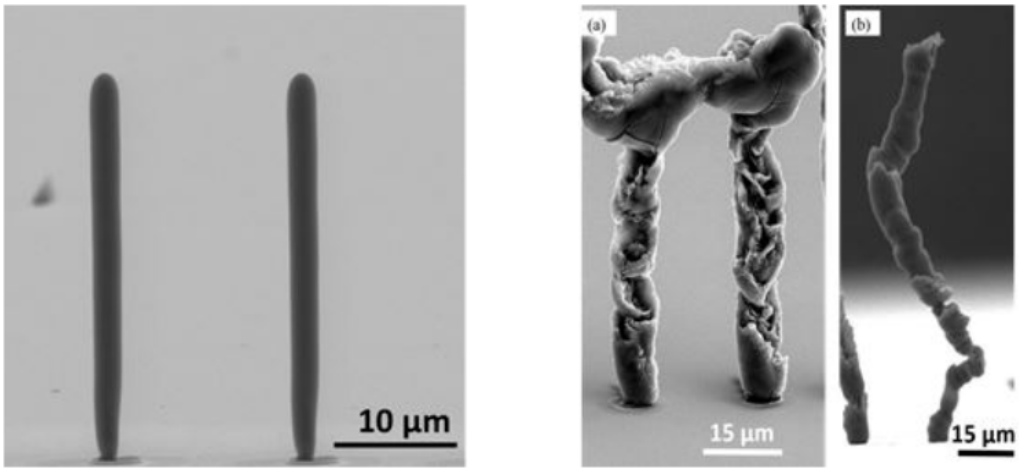

Figure 6. *Structure instability during slanted printing.*

In order to solve this issue, a double-axis rotating stage was developed to allow the immobilized nanoparticle liquid to be rotated to any orientation in all three dimensions (Figure 7). This system is composed of two cage motors and a clamp. A casing for these glass slides was designed using SolidWorks and printed in-house in order to prevent the glass slide from falling off during rotations (extending to 180 degrees). A LabView program was then written to calculate the degree of rotation for both axes and to control the rotating stage to create more complex shapes. By rotating the stage, the "pillar" remains parallel to gravity and does not become unstable or irregular.

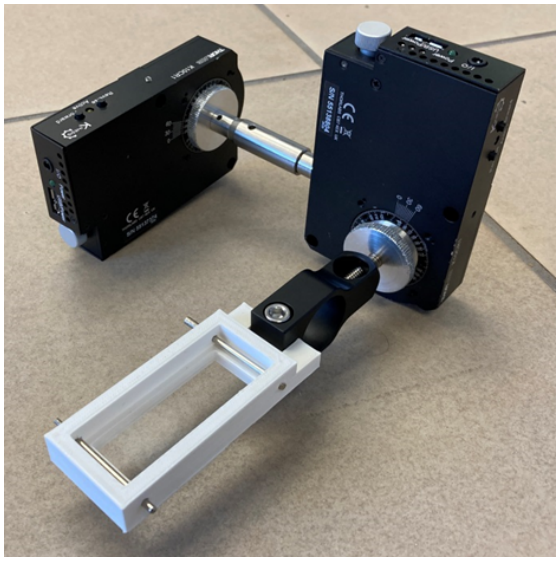

Figure 7. *Double-axis rotating cages.*

Conclusion

Understanding 3D technology and the tools to produce 3D structures on a nanoscale is critical for applications in many fields. Such implementation can allow for compact, stacked computer chips, precision micro-motors, and micro-probes. In addition, this technology can allow for stacking multiple electrical connections by alternating between conductors and insulators.[8,9] The unique combination of micro/nano-size and high precision will likely also lead to unanticipated, novel applications.

Clearly, as the nano-printing process develops, several questions require attention. For example, what other parameters affect wall regularity? What advantage could modulation of the laser intensity have over changing the stage speed? Would other frequencies present better walls? The next immediate step in the project is to print longer walls to see if some periodicity presents itself as we print even longer wall lengths (refer to Figure 3).

The subsequent step in the vector printing project is to combine the program of the cage motors with the program of the microscope stage. This system integration allows for both translation of the stage and rotation of the cage, resulting in an even wider range of motion. Another portion of this project is to finish the calculations for which rotation speeds correlate to which shape curves. Thinking even further ahead, problems such as the most efficient way of sending construction information to the printer arise, since as structural complexity increases, the programming instructions will also become more advanced. In some cases, it may remain efficient to transmit printing instructions in real-time. However, in other cases, it will likely be more efficient to program and compile the printing instructions separately in advance so they can be sent as a complete package for manufacturing. Other elements of the printing process will likewise also develop in response to the overall demand for optimization.

As the mechanical functioning of 3D nanotechnology develops to the point of applications, it lends itself to the biotechnology industry specifically and the medical industry in general. The pharmaceutical industry can utilize this 3D printing to manufacture precision pill carriers that allow for a controlled release of multiple medication ingredients.[10] Nano-printing can be utilized to produce medical and dental implants, and it can provide much more accurate surgical guides.[11] Material fabrication on the nanoscale benefits burn victims or plastic surgery patients as it creates a bi-layer composite for tissue regeneration. A 3D-printed micro-swimmer can explore the body at a cellular level, either gathering data or delivering products such as drugs. The precision required in chemotherapy improves when able to work at the individual cell level. Another benefit within the oncology field is using the 3D nano-printing technique to form bone scaffolding as a base for rebuilding and regeneration following treatment for bone diseases such as osteosarcoma.[3] This project, exploring layer-by-layer vs. vector printing techniques, has the potential to act as a launching pad for the exploration of critical steps in the process of three-dimensional nanoparticle printing.

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[12] Assistance from Eitan Edri on graphics

Yeshiva University Journal of Medicine and Dentistry

Research

iPLA2-6A Knockout Gene Partially Rescued Using *UAS-iPLA2-6A* Transgene in the Muscle Cells of *Drosophila melanogaster*

Yosef Scher

Abstract

Muscle cells play a critical role in the locomotor ability of an organism. While neurons send electrical signals via neurotransmitters to an organism's muscle cells to tell that muscle to move, it is ultimately the muscle cells themselves that conduct the movement through contraction and relaxation. Since many neurodegenerative diseases, such as Parkinson's disease, present with locomotor problems, muscle cells were specifically scrutinized. More specifically, *iPLA2-6A* functionality in muscle cells of *Drosophila melanogaster* to maintain aging health was investigated. In order to determine this, the wild-type allele was placed in mutant fly groups through the transgene, *UAS-iPLA2-6A*, to ascertain if the wild-type allele was sufficient to rescue motor decline in knockout *iPLA2-6A* flies. Climbing assays were performed to test if locomotor ability in the flies improved with the wild-type allele present. The 15-day climbing assay results suggested that there was a partial rescue of the *iPLA2-6A* gene in the mutant fly groups. However, this conclusion could not be definitively made, as $p = 0.084$ was slightly higher than an acceptable p-value. Unlike the 15-day climbing assay data, the 20-day climbing assay results revealed that partial rescue of the *iPLA2-6A* gene in the mutant fly groups was accomplished. The results were statistically significant, as $p = 0.020$. A comparison of the 15 and 20-day climbing assay results displayed a decrease in climbing index scores for both the control and mutant fly groups. That being said, the control fly group had a more significant reduction in climbing index scores than the mutant fly groups as the flies aged. Thus, partial rescue of the *iPLA2-6A* gene in the mutant fly groups was more apparent with time.

Introduction

Neurodegenerative diseases are a classification of diseases characterized by progressive dysfunction of neurons, synapses, glial cells, and their networks.[1] Although there are over a hundred documented neurodegenerative diseases known to date, including Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis (ALS), Parkinson's disease has become the most prevalent and fastest-growing neurodegenerative disease in the world.[2,3] From 1990 to 2015, 6.2 million people were diagnosed with Parkinson's disease—a 118% increase in global Parkinson's disease cases.[4] As global populations continue to age, the primary factor that has been proven to be a cause of neurodegenerative disease, Parkinson's cases are predicted to

increase exponentially.[2,5] By 2040, researchers predict that the number of Parkinson's cases will double to 12 million documented cases.[2]

Even though most Parkinson's cases are sporadic, believed to be caused by a combination of genetic and environmental factors, the less common inherited familial parkinsonism has allowed researchers to identify the various loci attributed to Parkinson's disease when the loci are perturbed.[6] By gaining a more advanced understanding of the underlying molecular and cellular mechanisms that lead to the rarer inherited familial parkinsonism, scientists hope to apply their findings to the more frequent sporadic cases of Parkinson's. In order to investigate human familial parkinsonism, orthologous genes to human familial parkinsonism loci were mutated in *Drosophila melanogaster*. One such orthologous gene, *iPLA2-6A*, has been shown to be a disease locus for various neurodegenerative diseases, including a rare inherited familial parkinsonism called autosomal recessive dystonia-parkinsonism.[7]

By examining the molecular effects of the *iPLA2-6A* mutation, researchers have garnered a better etiological understanding of Parkinson's disease. In a recent journal article by Mori et al., two novel conclusions revealed how the *iPLA2-6A* mutation affected molecular mechanisms in organisms.[8] First, Mori et al. discovered that a lack of *iPLA2-6A* activity resulted in the shortening of phospholipid acyl chains.[8] This led to endoplasmic reticulum stress that affected neuronal activity in the flies. Second, the researchers found that α-Synuclein, a presynaptic neuronal protein that is linked neuropathologically and genetically to Parkinson's disease, is aided by phospholipids with shorter acyl chains.[8,9] With these two pieces of information, Mori et al. proposed a hypothesis as to how neurodegeneration occurs in flies with the loss of the *iPLA2-6A* gene and affects their brains at the molecular level (Figure 1). When the *iPLA2-6A* gene was removed, phospholipid acyl chains in the brain were shortened. As a result, the shortened phospholipid acyl chains had altered curvature, membrane fluidity, and lipid packing abilities. One of the effects of the altered phospholipid properties was α-Synuclein aggregation, which was caused by α-Synuclein's decreased ability to bind to the synaptic membrane effectively. Additional effects of the altered phospholipid properties were stress placed onto the endoplasmic reticulum and abnormal functionality of neurotransmitters.

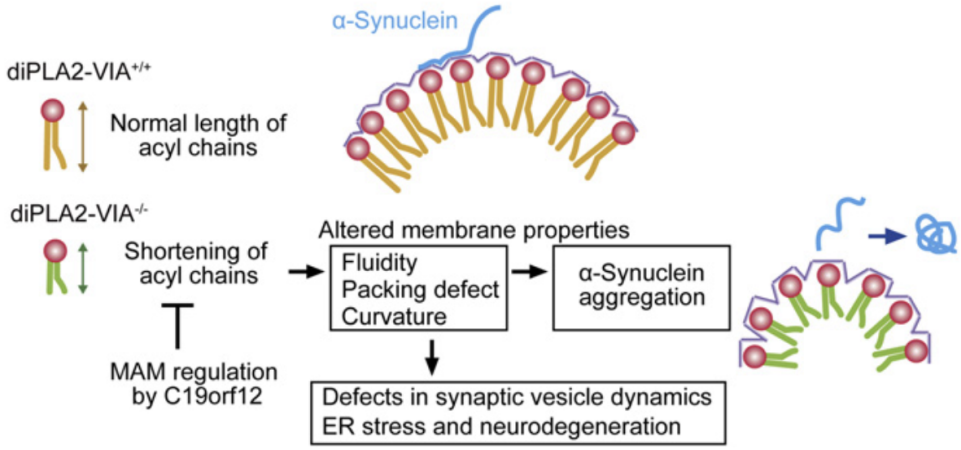

Figure 1. Mori et al. Hypothesis of the Molecular Effects of the *iPLA2-6A* Gene Removal.[8] *When the iPLA2-6A gene was removed, phospholipid acyl chains in the brain were shortened. This caused the shortened phospholipid acyl chains to have altered curvature, membrane fluidity, and lipid packing abilities. One of the effects of the altered phospholipid properties was α-Synuclein aggregation, which was caused by α-Synuclein's decreased ability to bind to the synaptic membrane effectively.*

Previous research investigating the effects of knocking out the *iPLA2-6A* gene has demonstrated that neurodegeneration occurs in flies and that rescue of the *iPLA2-6A* gene in tissue-specific cells is possible. In 2021, Steinhauer et al. published a paper that illustrated that *Drosophila melanogaster* null *iPLA2-6A* mutants exhibited a progressive loss of locomotor ability with age, consistent with neurodegeneration.[10] Using an RNAi knockdown in specific neuronal subsets and muscle cells, the locomotor defect was phenocopied. Control and mutant flies performed standard climbing assays to test for locomotor defects at fifteen and twenty days after eclosion. It was found that certain subsets of cells, such as muscle and pan-neuronal cells, were rescued with the *DJ667-GAL4* and *elav-GAL4* phenocopies. A critical takeaway from the research was that *iPLA2-6A* is required in muscles and neurons in order for the flies to maintain normal locomotor skills as they age.[10]

While extensive research has been conducted on the effects of removing the *iPLA2-6A* gene from specialized cells, such as neurons and muscle cells, no known research has been conducted to investigate in which specific subset of cells *iPLA2-6A* functions to maintain aging health. To elucidate an answer, wild-type *iPLA2-6A* expression in neurons, specific subsets of neurons, and muscle cells were used to examine if the wild-type allele was sufficient to rescue motor decline seen in *PLA2G6* Associated Neurodegeneration (PLAN) disorders, such as autosomal recessive dystonia-parkinsonism.

Methods

Fly Crosses

Fly strains were provided by outside researchers unbeknownst to the experimenter. In order to establish fly strains that enabled gene expression only in muscle cells, a *GAL4-UAS* system was required. *GAL4* is a transcriptional activator that binds to a *UAS* enhancer sequence found in an organism's DNA.[11] The *GAL4-UAS* system recruits transcription machinery to a specific locus for gene expression to occur. This causes any genes downstream of the *UAS* sequence to just be expressed when *GAL4* is expressed. Therefore, a fly strain expressing *GAL4* with a particular promoter can cause tissue-specific expression.[11] To restore the wild-type *UAS-iPLA2-6A* only in muscle cells, a specific *GAL4* driver, *GAL4-DJ667*, was needed. The fly crosses made for the muscle cell group, representing the parental generation, are written out in Punnett Squares 1 and 2.

Punnett Square 1. Experimental (Mutant) Group Fly Cross*

	GAL4-DJ667	Stubble
UAS-iPLA2-6A	*UAS-iPLA2-6A*/*GAL4-DJ667*	*UAS-iPLA2-6A*/Stubble
Curly	Curly/*GAL4-DJ667*	Curly/Stubble

Punnett Square 2. Control Group Fly Cross*

	GAL4-DJ667	Stubble
+	+/*GAL4-DJ667*	+/Stubble
Curly	Curly/*GAL4-DJ667*	Curly/Stubble

* Note: The crosses were maintained at 26 ºC. The highlighted cells represent the genotypes of the flies that needed to be collected. The phenotype of these flies was non-curly wings and non-stubble hairs located on the thorax of the fly. Additionally, only male flies were collected to ensure that no F_2 progeny arose from matings between F_1 flies.

Collecting and Sorting the F_1 Progeny

After the initial crosses were made, ten to twelve days passed before the F_1 flies eclosed and were ready to be sorted and collected. The flies were first gassed with a small amount of carbon dioxide so that they could be transferred to a platform emitting carbon dioxide. Using a microscope, flies with the desired phenotype of male, non-curly, and non-stubble were sorted and collected every two to three days for approximately two weeks. A group of 6–13 flies with the desired phenotype was placed into a vial until the 15 and 20-day climbing assays were conducted. Each new group of flies for the control group was marked C_x and E_x for the

experimental group, where "x" represents the group number used in the climbing assay. From October 24th to November 28th, 15 control groups and 14 experimental groups were created every 2–3 days, holding 6–13 F_1 flies in a vial. While the number of flies varied in each vial, 115 flies from the control group and 108 flies from the experimental group were collected over the approximate month time period.

Passing the Flies

In addition to collecting and sorting the flies every 2–3 days, the F_1 flies placed in vials to age needed to be passed 3–5 days either because they required more food or the food was receding from the wall. In order to pass the flies, two techniques were utilized. One technique used small amounts of carbon dioxide to gas the F_1 flies so that they could be transferred to a new, fresh vial of food. The other technique involved transferring the flies without the use of carbon dioxide; the old food vial with a cotton stopper in it would be tapped on a mouse pad continuously to ensure that no flies escaped while the cotton stopper was removed, allowing the new food vial to be placed on top of it. The food vial would be flipped, and the flies would be transferred to the new vial of fresh food.

Climbing Assays

At the 15 and 20-day mark of the F_1 flies being in their respective vials, climbing assays were performed. Climbing assays are tests that measure the locomotor ability of the flies. After the flies were transferred to a new food vial, a second glass vial was taped to the food vial containing the flies. A ruler measured 6 cm from the edge of the food, acting as the threshold line that the flies needed to pass to be counted as having sufficient locomotor ability. When the climbing assay apparatus was all setup, the climbing assay test began (Figure 2).

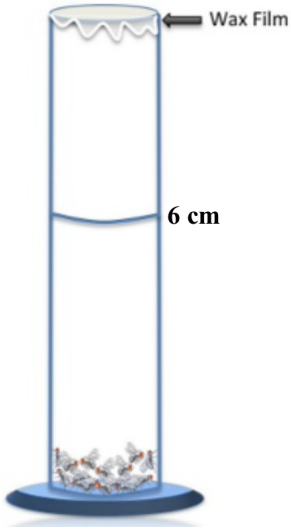

Figure 2. Climbing Assay Apparatus (Adapted from Reference 12). *The climbing assay apparatus consisted of two plastic vials stacked on top of each other. 6 cm from the edge of the food (not shown) was measured with a ruler. A marker was used to mark the 6 cm threshold line. A cotton stopper was placed at the top of the climbing apparatus so the flies would not escape.*

One person tapped the apparatus on a mouse pad for 1–2 seconds before letting go of it, as the second person started the stopwatch to see how many flies crossed the 6 cm threshold in 20 seconds. The climbing assay test was performed five times per fly vial. Each time, the number of flies that crossed the 6 cm threshold was recorded. When the five trials for a group were completed, the climbing index was calculated. The climbing index was calculated by summing the total score of all the flies from the five trials that crossed the 6 cm threshold and dividing that by the number of flies in a vial. The minimum score a vial could receive was 0.0, and the maximum was 5.0. Climbing indices were averaged for the 15 and 20-day climbing assays for the control and experimental groups. The climbing indices were plotted with standard deviations. Climbing indices for each condition were verified for normal distribution around the average. Statistical comparisons were performed using unpaired t-tests.

Statistical Tests

An unpaired t-test was used to compare the average between the two independent groups, i.e., control and mutant fly groups, to test whether a significant difference was present between the two groups. Several assumptions were made when performing the unpaired t-test.[13] First, it was assumed that there was equal variance between the control and mutant fly groups. If equal variance exists between the two groups, then the data from the two groups should have the same standard deviation. Second, two independent groups were needed in order to conduct the unpaired t-test. Finally, observations between the control and mutant fly groups were required to be sampled independently.

Results

Raw Climbing Index Data

The 15 and 20-day climbing assay data for the control group collected from October 24th to November 28th are shown below in Figures 4 and 5, respectively. Five climbing assay trials were performed for each F_1 fly control group, which were then used to calculate the climbing indices of each group. A total of 115 male flies that varied in number between the 15 control groups had an average climbing index of 3.25 with a standard deviation of 1.50 for the 15-day climbing assay and an average climbing index of 1.88 with a standard deviation of 1.36 for the 20-day climbing assay (Figure 3).

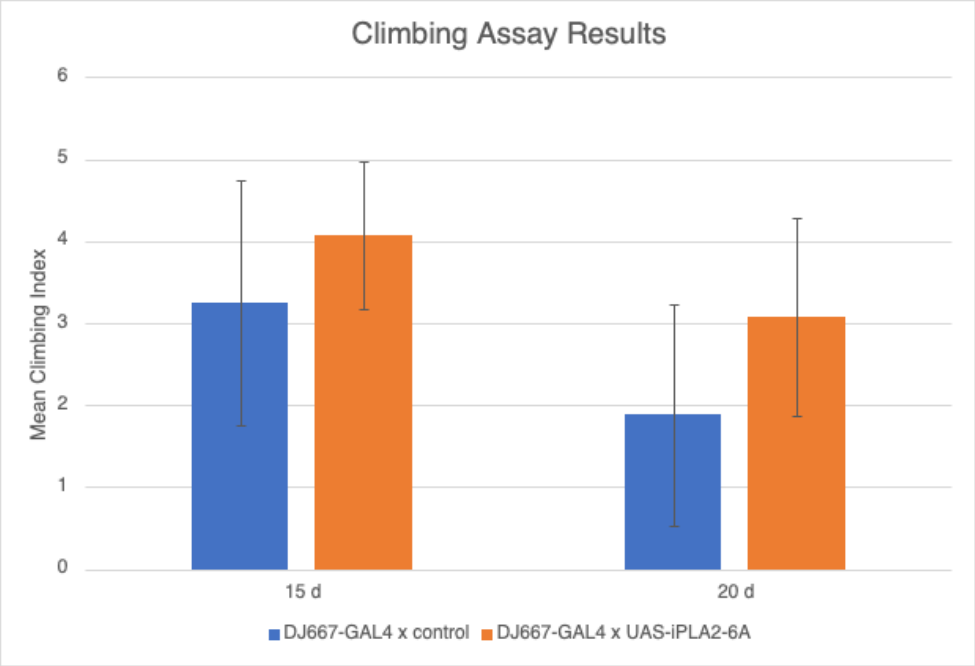

Figure 3. Graphical Representation of the Mean Climbing Index Plotted with the Standard Deviation. *The results for the 15 and 20-day climbing assays are illustrated in the graph. The blue bars represent the average climbing index score for the control male F_1 flies, and the orange bars represent the average climbing index score for the mutant male F_1 flies. The error bars represent the standard deviation of the datasets. It should be noted that 8 out of the 58 climbing assays performed were done 1–2 days before or after the planned climbing assay day, which could have potentially skewed the average climbing index.*

15 d									
Group	Date collected	Date tested	N Flies	T #1	T #2	T #3	T #4	T #5	CI
C1	26-Oct	10-Nov	5	3	5	5	5	5	4.60
C2	26-Oct	10-Nov	6	6	4	5	5	4	4.00
C3	31-Oct	16-Nov	7	6	6	5	6	5	4.00
C4	31-Oct	16-Nov	6	5	5	5	5	5	4.17
C6	2-Nov	17-Nov	10	7	7	6	6	7	3.30
C7	2-Nov	17-Nov	9	7	7	6	4	5	3.22
C8	6-Nov	22-Nov	6	0	0	2	3	1	1.00
C9	6-Nov	22-Nov	6	0	0	1	0	0	0.17
C10	9-Nov	28-Nov	8	5	7	8	6	8	4.25
C11	13-Nov	28-Nov	8	6	3	7	6	3	3.13
C12	17-Nov	1-Dec	6	1	1	1	0	0	0.50
C13	20-Nov	5-Dec	9	6	8	7	8	7	4.00
C14	22-Nov	7-Dec	5	5	5	5	5	5	5.00
C15	27-Nov	12-Dec	14	12	12	11	11	12	4.14
C16	28-Nov	13-Dec	10	7	5	6	7	8	3.30
Total N flies			115					AVG	3.25156
Number of groups			15					SD	1.49674

Figure 4. Raw Data for 15-day Climbing Indices (CI) of the Control Group. *A total of 115 male flies comprised the 15 groups of F_1 control flies. Climbing indices for the 15 groups of F_1 control flies had anywhere from 5–14 flies in a group, represented by "N Flies," were calculated. "T#X," where "X" represents a number 1–5, was the trial number of how many flies crossed the 6 cm threshold to be counted as a successful climb. It should be noted that group C10's climbing assay was conducted on day 19 instead of day 15. The mean climbing index was determined to be approximately 3.25, with a standard deviation of approximately 1.50.*

20 d								
Group	Date tested	N Flies	T #1	T #2	T #3	T #4	T #5	CI
C1	16-Nov	5	1	1	1	1	1	1.00
C2	16-Nov	6	0	0	0	0	1	0.17
C3	20-Nov	7	3	3	2	2	3	1.86
C4	20-Nov	6	3	4	4	4	4	3.17
C6	22-Nov	10	7	8	7	7	7	3.60
C7	22-Nov	8	4	3	6	4	3	2.50
C8	28-Nov	6	1	3	2	1	1	1.33
C9	28-Nov	6	2	1	4	2	1	1.67
C10	1-Dec	8	0	0	0	0	1	0.13
C11	1-Dec	8	5	6	5	7	6	3.63
C12	7-Dec	5	4	3	2	3	2	2.80
C13	10-Dec	8	1	2	2	2	1	1.00
C14	12-Dec	5	4	5	4	4	4	4.20
C15	17-Dec	14	4	2	3	2	3	1.00
C16	18-Dec	10	0	0	0	1	1	0.20
Total N flies			112				AVG	1.8827
Number of groups			15				SD	1.35694

Figure 5. Raw Data for 20-day Climbing Indices (CI) of the Control Group. *A total of 112 male flies comprised the 15 groups of F_1 control flies. Fewer flies performed climbing assays because some flies were lost, transferring the flies to fresh food vials. Climbing indices for the 15 groups of F_1 control flies had anywhere from 5–14 flies in a group, represented by "N Flies," were calculated. "T#X," where "X" represents a number 1–5, was the trial number of how many flies crossed the 6 cm threshold to be counted as a successful climb. It should be noted that groups C9 and C10's climbing assays were conducted on day 22 instead of day 20. Additionally, group C11's climbing assay was conducted on day 18 instead of day 20. The mean climbing index was determined to be approximately 1.88, with a standard deviation of approximately 1.36.*

The 15 and 20-day climbing assay data for the experimental group collected from October 24th to November 28th are shown below in Figures 6 and 7, respectively. Five climbing assay trials were performed for each F_1 fly experimental group, which were then used to calculate the climbing indices of each group. A total of 108 male flies that varied in number between the 14 experimental groups had an average climbing index of 4.08 with a standard deviation of 0.907 for the 15-day climbing assay and an average climbing index of 3.08 with a standard deviation of 1.21 for the 20-day climbing assay.

15 d									
Group	Date collected	Date tested	N Flies	T #1	T #2	T #3	T #4	T #5	CI
E2	26-Oct	10-Nov	7	7	7	7	7	7	5.00
E3	31-Oct	15-Nov	6	6	5	6	5	6	4.67
E4	31-Oct	17-Nov	9	7	7	7	7	7	3.89
E5	31-Oct	17-Nov	6	5	6	5	5	6	4.50
E6	2-Nov	17-Nov	10	8	7	7	8	9	3.90
E8	6-Nov	22-Nov	9	8	7	8	8	9	4.44
E9	6-Nov	22-Nov	9	8	7	6	5	8	3.78
E10	9-Nov	28-Nov	7	6	7	7	7	6	4.71
E11	13-Nov	28-Nov	7	6	6	7	6	7	4.57
E12	17-Nov	1-Dec	6	2	2	2	2	2	1.67
E13	17-Nov	1-Dec	7	3	4	5	4	3	2.71
E14	20-Nov	5-Dec	8	8	8	8	7	8	4.88
E15	27-Nov	12-Dec	9	7	8	8	8	8	4.33
E16	28-Nov	13-Dec	8	8	8	5	6	5	4.00
Total N flies			108					AVG	4.0752
Number of groups			14					SD	0.90705

Figure 6. Raw Data for 15-day Climbing Indices (CI) of the Experimental Group. *A total of 108 male flies comprised the 14 groups of F_1 experimental flies. Climbing indices for the 14 groups of F_1 experimental flies had anywhere from 6–10 flies in a group, represented by "N Flies," were calculated. "T#X," where "X" represents a number 1–5, was the trial number of how many flies crossed the 6 cm threshold to be counted as a successful climb. The mean climbing index was determined to be approximately 4.08, with a standard deviation of approximately 0.907.*

20 d								
Group	Date tested	N Flies	T #1	T #2	T #3	T #4	T #5	CI
E2	15-Nov	5	4	2	3	3	2	2.80
E3	20-Nov	5	5	5	5	3	4	4.40
E4	20-Nov	8	6	5	5	6	5	3.38
E5	20-Nov	6	6	6	6	6	6	5.00
E6	22-Nov	10	3	0	5	2	0	1.00
E8	28-Nov	8	5	7	7	6	6	3.88
E9	28-Nov	9	6	7	8	9	5	3.89
E10	29-Nov	7	1	1	4	4	3	1.86
E11	1-Dec	7	4	6	5	6	5	3.71
E12	7-Dec	6	3	3	4	3	2	2.50
E13	7-Dec	6	4	5	5	5	5	4.00
E14	10-Dec	8	5	7	6	5	4	3.38
E15	17-Dec	9	1	3	1	2	3	1.11
E16	18-Dec	8	5	3	4	3	3	2.25
Total N flies			102				AVG	3.08189
Number of groups			14				SD	1.20703

Figure 7. Raw Data for 20-day Climbing Indices (CI) of the Experimental Group. *A total of 102 male flies comprised the 14 groups of F_1 experimental flies. Fewer flies performed climbing assays because some flies were lost, transferring the flies to fresh food vials. Climbing indices for the 14 groups of F_1 experimental flies had anywhere from 5–10 flies in a group, represented by "N Flies," were calculated. "T#X," where "X" represents a number 1–5, was the trial number of how many flies crossed the 6 cm threshold to be counted as a successful climb. The mean climbing index was determined to be approximately 3.08, with a standard deviation of approximately 1.21.*

Unpaired t-test Results

The results of the unpaired t-tests for the control and experimental groups are shown in Table 1. The 15-day climbing assay unpaired t-test revealed that the results were not statistically significant, indicated by the p-value being greater than 0.05. In contrast, the 20-day climbing assay unpaired t-test demonstrated that the results were statistically significant. This was proven by the p-value being less than 0.05.

Table 1. P-Values for the 15 and 20-day Climbing Assays

15-Day Climbing Assay P-Value	20-Day Climbing Assay P-Value
$p = 0.084$	$p = 0.020$

Discussion

15-Day Climbing Assay Data Analysis

An analysis of the data amassed for the 15-day climbing assay suggested that rescue of the *iPLA2-6A* gene occurred, but this conclusion could not be corroborated since the p-value for the unpaired t-test was not statistically significant. That being said, the p-value was near the standard $p < 0.05$ value for the results from this data to be considered significant and not due to chance. It is likely that with a larger sample size and additional 15-day climbing assays performed on new fly groups, the results would yield a statistically significant value.[14] Further research must be conducted to test this hypothesis. Not surprisingly, the mutant male F_1 flies had a higher climbing index than the control F_1 male fly group. This was expected since rescue was more able to occur in the mutant male flies, helping them potentially be more successful at climbing than in the control fly group. The standard deviation was much higher than expected. Possible explanations for why the data varied so much from the mean include the time of day the climbing assays were conducted and unequal tapping strength applied to the climbing assay apparatus by different members in the lab group, leading to variable results. Reiger et al. found that fruit flies were most active at early dawn and late dusk.[15] At the beginning of the little over a month time period spent collecting climbing index data, many of the climbing assays performed were conducted at late dusk. Interestingly, the highest climbing indices were recorded at that time of day. Therefore, climbing assays performed at times not at early dawn or late dusk may be the cause for the variable data, leading to a high standard deviation. However, once again, increasing the sample size should alleviate the high standard deviation, but further testing is needed to validate this claim.[16]

20-Day Climbing Assay Data Analysis

The data collected for the 20-day climbing assay was determined to be statistically significant, as the p-value for the unpaired t-test was 0.020. The mutant F_1 flies had a significantly better climbing index score than the F_1 control fly groups. This was expected since rescue was more able to occur in the mutant male flies, helping them potentially be more successful at climbing than in the control fly groups. As expected, the climbing index score for both the control and mutant flies of the 20-day climbing assays was lower than the climbing index score for the control and mutant flies of the 15-day climbing assays. Since more time passed, the 20-day control and male mutant flies had less locomotor ability than they did 5 days prior. Furthermore, as predicted, the control fly groups from the 15 and 20-day climbing assays had a more profound loss of successful climbs compared to the male mutant flies. As a result, the average climbing indices for the control fly groups had a 42.1% reduction in successful climbs (Calculation 1).

Compared to the control fly groups, the average climbing indices for the male mutant fly groups were only reduced by 24.4% (Calculation 2).

$$Calculation\ 1.\ \ \%\ Decrease = \frac{Final - Starting\ Value}{|Starting\ Value|} x\ 100\% \rightarrow \frac{1.88 - 3.25}{|3.25|}\ x\ 100\% = -42.1\%$$

$$Calculation\ 2.\ \ \%\ Decrease = \frac{Final - Starting\ Value}{|Starting\ Value|} x\ 100\% \rightarrow \frac{3.08 - 4.08}{4.08}\ x\ 100\% = -24.4\%$$

Like the 15-day climbing assay data, the standard deviation for the 20-day climbing assay data was higher than expected. This could be due to the reasons proposed in the 15-day climbing assay data analysis section or some other reasons yet to be determined. In any case, to make the data less varied from the mean, further research should be conducted where the sample size of the flies is larger than the one used here.[16]

Examining the Molecular Mechanisms that Led to Rescue in the Mutant Fly Groups

A thorough explanation of the molecular mechanisms helps one to understand why the wild-type *iPLA2-6A* allele was able to be more rescued in the muscle cells of the mutant male fly groups than the control fly groups. As mentioned earlier, a specific *GAL4* driver, *GAL4-DJ667*, was required to restore the wild-type allele for the *iPLA2-6A* gene only in muscle cells. In order to accomplish this, *GAL4-DJ667* was bound with *UAS-iPLA2-6A* (Figure 8).

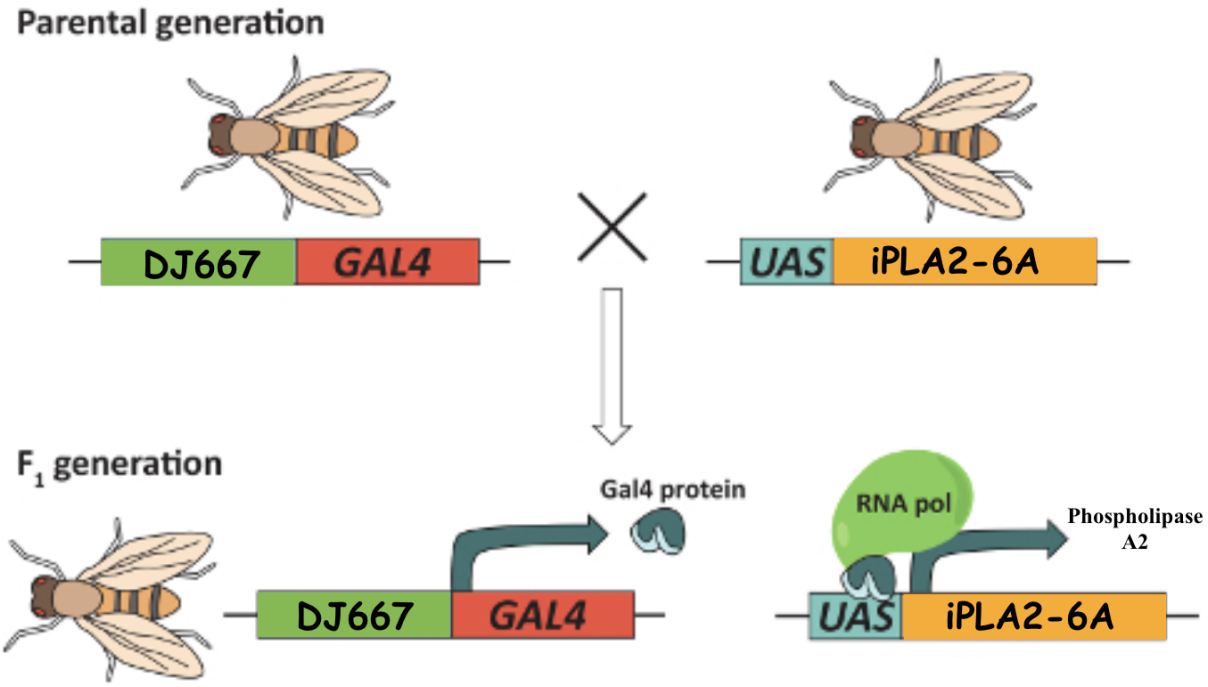

Figure 8. Molecular Representation of How Gene Expression Only Occurred in the Muscle Cells of the Mutant Flies (Adapted from Reference 17). *In order to express the iPLA2-6A gene only in the muscle cells, a fly with DJ667-GAL4 was mated to a fly with the UAS-iPLA2-6A transgene. The F_1 progeny's genotype thus became DJ667-GAL4/UAS-iPLA2-6A. A GAL4 protein that acts as a transcriptional activator binds to the UAS-iPLA2-6A transgene. When RNA polymerase binds to the GAL4 protein, Phospholipase A2, an enzyme that helps with lipid metabolism and maintaining the integrity of the cell membrane, is produced and expressed.*[18] *Any genes that are encoded downstream of the UAS-iPLA2-6A transgene will only be expressed when GAL4 is expressed.*[11]

The *DJ667-GAL4*/*UAS-iPLA2-6A* system expressed the wild-type allele of the *UAS-iPLA2-6A* transgene in the mutant fly groups but not in the control fly groups since the control fly groups lacked the *UAS-iPLA2-6A* transgene. As such, Phospholipase A2, the gene product of the *UAS-iPLA2-6A* transgene, was not able to be produced and expressed. Phospholipase A2 is an important enzyme that assists with lipid metabolism and maintaining the integrity of the cell membrane.[18] As Mori et al. hypothesized, the lack of integrity of the cell membrane caused α-Synuclein to aggregate in the form of Lewy bodies and Lewy neurites.[8,19] An overabundance of α-Synuclein has been shown to lead to neuronal death, a defining feature of neurodegenerative diseases like Parkinson's disease.[9] Specifically related to Parkinson's disease, α-Synuclein has also been proven to regulate dopamine production.[9] When α-Synuclein accumulates, dopamine production is inhibited. Since dopamine normally inhibits α-Synuclein aggregation, Lewy bodies and Lewy neurites, large clumps of α-Synuclein, continue to accumulate in an organism's brain.

When a loss of function mutation occurred in the *iPLA2-6A* gene of the control group flies, Phospholipase A2 was not produced, and so the integrity of the cell membrane was not able to be maintained. This led to an accumulation of α-Synuclein in the brains of the flies, which resulted in them developing Parkinson's disease. The locomotor ability of the control fly group was hindered, as seen by the lower climbing index scores compared to the mutant fly groups. Like the control fly groups, the mutant fly groups initially also had a loss of function mutation in the *iPLA2-6A* gene. This caused the flies to have an accumulation of α-Synuclein in their brains, which led to Parkinson's disease. However, after establishing crosses that yielded F_1 fly progeny with the *UAS-iPLA2-6A* transgene, Phospholipase A2 was able to be produced once again. Consequently, the mutant fly groups performed significantly better in the climbing assay tests than the control fly groups.

The results of this experiment have proven that muscle cells are affected by Parkinson's disease, especially as time passes, and that rescue can occur in tissue-specific cells. Future research should focus on investigating if tissue-specific rescue can be achieved in humans who have developed Parkinson's disease.

Future Experimentation: Adaptation of FlyVRena to Further Investigate Rescue of the iPLA2-6A Gene, Leading to Better Locomotor Ability

FlyVRena, a device created by Cruz et al., can be adapted to examine how rescue of the *iPLA2-6A* gene can lead to better locomotor ability in flies.[20] The analyzed results from the FlyVRena experiment will be compared to the results of the flies' locomotor ability during climbing assays.

FlyVRena is a virtual reality system that allows insects to walk freely in an arena, all the while sensors and cameras collect and analyze data on the fly's movement (Figure 9).

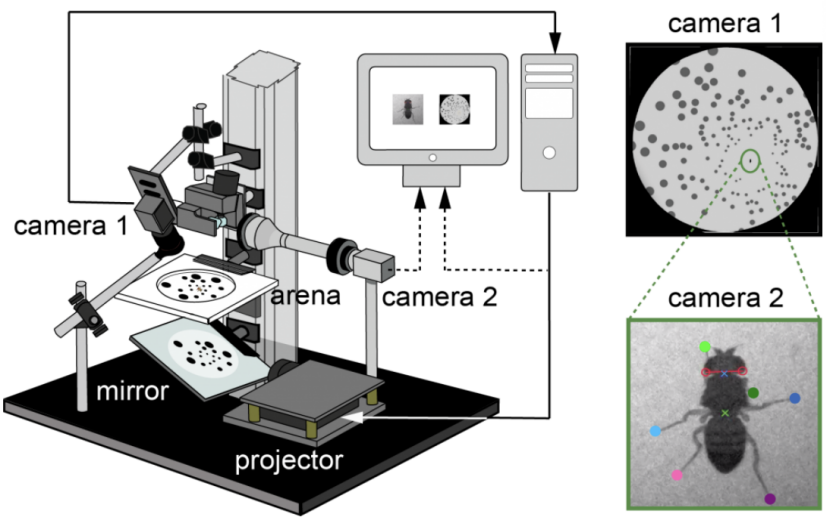

Figure 9. FlyVRena.[20] *A fruit fly is placed in the arena and is allowed to roam the arena floor. Cameras and sensors track the movement of the fly and collect and analyze data based on the fly's movement. The data that is collected and analyzed is sent to a computer, where the data can be further analyzed with specific software. While the figure does not show it, the top of the arena is enclosed with a plate of glass covered in sigmacote, a chemical that the flies do not like. This deters the flies from walking on the ceiling.*

While the original experiment conducted by Cruz et al. used virtual reality to make objects appear as the flies roamed the arena floor, the use of virtual reality will not be required in this future experiment.[20] As such, the projector and mirror parts of FlyVRena would not be used. However, the other components of FlyVRena, including the arena, stand to hold the arena, cover of the arena coated with a layer of sigmacote, camera, sensors, and a computer to further analyze the data, would be used.

The climbing assays performed in this experiment tested one form of locomotion that the flies could perform: flying. However, flies have other locomotor abilities, such as walking. By using FlyVRena, the walking abilities of the flies would be examined. New control and mutant fly groups that had the *iPLA2-6A* gene knocked out would be used. The same procedure in terms of passing, collecting, and sorting the flies from both groups would be followed. When the F_1 flies from the control and mutant fly groups reached 15 and 20 days old, a single fly from the control and mutant groups would be placed in a FlyVRena apparatus. In order to ensure that the flies would walk only on the floor of the arena, certain modifications would need to be made to the apparatus. To start, since flies have been shown to be phobiocentric, the circumference of the arena would need to be heated to a temperature of 40 ºC. This would ensure that the flies stayed toward the middle of the arena. Additionally, to ensure that the flies stay on the floor of the arena

and not roam on the ceiling, a cover with a thin layer of sigmacote, a chemical that the flies are averse to, would be placed on top of the arena. With these modifications in place, the fly would be forced to walk on the floor of the arena.

The flies would have a 10-minute exploring period so that they could acclimate to their new environment. When the 10 minutes passed, the fly would be allowed to walk the arena for 30 minutes. Cameras and sensors would track the movement of the fly and send this information to the computer, where software can be used to analyze the data. This procedure would be done for five trials. The walking index, which is the total distance covered by all the flies in a group divided by the total number of flies in a group, would then be calculated. It is predicted that the control fly groups would explore a lot less of the arena than the mutant fly groups since locomotor ability in the control fly groups should be more impeded as rescue of the *iPLA2-6A* gene is not occurring (Figure 10). This would be shown statistically by the control fly groups having a lower walking index than the mutant fly groups. The data from the FlyVRena experiment would be compared to the data from the climbing assay experiment to examine if certain forms of locomotion seem to have better rescue than the other forms of locomotion.

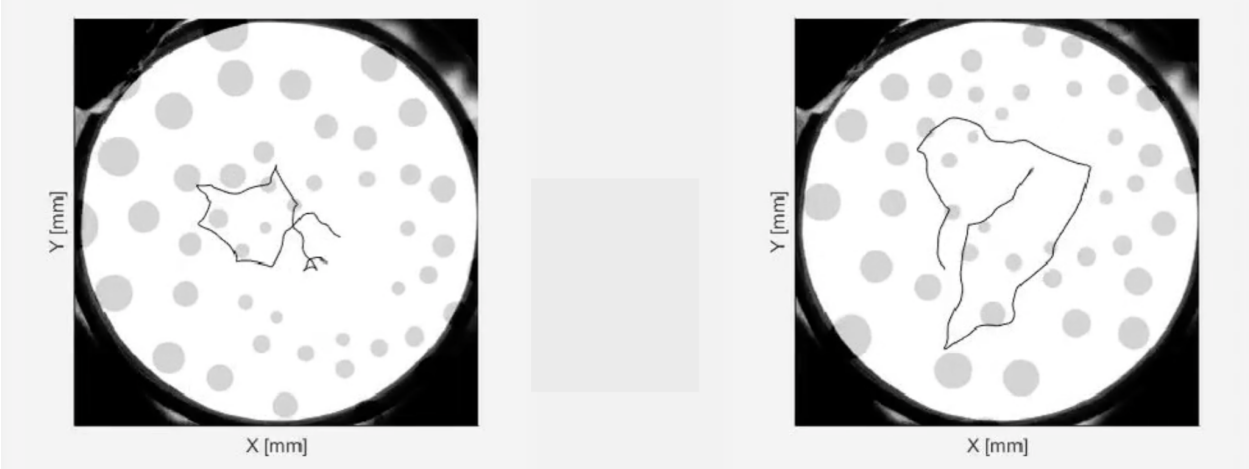

Figure 10. Predicted Walking Patterns of the Control Fly Groups (Left) vs. the Mutant Fly Groups (Right).[20] *A fly would be placed in the FlyVRena and then be given a 10-minute exploratory period to acclimate the fly to its environment. Then, the fly would be given 30 minutes to walk around the arena. The movement of the fly would be tracked by cameras and sensors, and that data would be sent to a computer for analysis using software programs. It is predicted that the control fly groups would have a similar walking pattern to the picture on the left, as no rescue of the iPLA2-6A gene is occurring in those flies. As a result, Phospholipase A2 is not being produced, so the flies would accumulate α-Synuclein in their brains. This would lead to Parkinson's disease, which presents with reduced locomotor ability. In contrast, the mutant fly groups are predicted to have walking patterns similar to the picture on the right since rescue of the iPLA2-6A gene is occurring in only those flies. Phospholipase A2 is being produced, so the*

flies are not accumulating a large amount of α-Synuclein in their brains. This should lead to not as severe locomotor impediments as the control fly groups are predicted to have.

Conclusion

This experiment investigated if *iPLA2-6A* functions in muscle cells of *Drosophila melanogaster* to maintain aging health. In order to determine this, the wild-type allele was placed in mutant fly groups through the transgene, *UAS-iPLA2-6A*, to ascertain if the wild-type allele was sufficient to rescue motor decline in knockout *iPLA2-6A* flies. Climbing assays were performed to test if locomotor ability in the flies improved with the wild-type allele present. Although the 15-day climbing assay results suggested that there was a partial rescue of the *iPLA2-6A* gene in the mutant fly groups, this conclusion could not be definitively stated, as the p-value was slightly higher than an acceptable p-value. Unlike the 15-day climbing assay data, the 20-day climbing assay p-value revealed that partial rescue of the *iPLA2-6A* gene in the mutant fly groups was accomplished. A comparison of the 15 and 20-day climbing assay results displayed a decrease in climbing index scores for both the control and mutant fly groups. That being said, as the flies aged, the control fly groups had a more significant reduction in climbing index scores than the mutant fly groups. Thus, partial rescue of the *iPLA2-6A* gene in the mutant fly groups was more apparent with time. Future research should focus on investigating if tissue-specific rescue can be achieved in humans who have developed Parkinson's disease.

Acknowledgments

All fly data was conducted in Professor Josefa Steinhauer's laboratory, an associate professor of biology at Yeshiva University. I would like to thank her for her continuous support and assistance over the course of my research. Additionally, I would like to thank my laboratory partners, Aaron Lubat, Alexander Siegman, and Shlomo Shaulian, for their help with collecting data for this research project.

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Review

BRG1: Promoter or Suppressor of Cancer? The Outcome of BRG1's Interaction with Specific Cellular Pathways

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Abstract

BRG1 is one of two catalytic subunits of the SWI/SNF ATP-dependent chromatin-remodeling complex. In cancer, it has been hypothesized that BRG1 acts as a tumor suppressor. Further study has shown that, under certain circumstances, BRG1 acts as an oncogene. Targeted knockout of BRG1 has proven successful in most cancers in suppressing tumor growth and proliferation. Furthermore, BRG1 effects cancer proliferation in oncogenic *KRAS* mutated cancers, with varying directionality. Thus, dissecting BRG1's interaction with various cellular pathways can highlight possible intermediates that can facilitate the design of different treatment methods, including BRG1 inhibition. Autophagy and apoptosis are two important cellular responses to stress. BRG1 plays a direct role in autophagy and apoptosis and likely promotes autophagy and suppresses apoptosis, supporting unfettered cancer growth. PRMT5 inhibits transcription by interacting with ATP-dependent chromatin remodeling complexes, such as SWI/SNF. When PRMT5 associates with the SWI/SNF complex, including BRG1, it represses tumor suppressor genes. The Ras/Raf/MAPK/ERK1/2 pathway in cancers is a signal transduction pathway involved in the transcription of genes related to cancer survival. BRG1 has been shown to effect KRAS-driven cancer growth. BRG1 associates with several proteins within the signal transduction pathway. In this review, we analyze BRG1 as a promising target for cancer inhibition and possible synergy with other cancer treatments.

Introduction

Brahma-related gene-1 (BRG1) is utilized as a catalytic subunit by a variety of enzymatic complexes which modify chromatin structure.[1] *SMARCA4* (also referred to in this article as BRG1) is the gene encoding for BRG1 expression. Chromatin units restructured by such complexes containing BRG1 have been observed to both trigger and hinder gene expression. Additionally, BRG1 has proven to play a role in complexes that signal for gene silencing of certain promoter regions, thus repressing transcriptional function.[2] ATP hydrolysis provides the necessary energy for such chromatin remodeling complexes which contribute to the reformation of the nucleosome.[3]

BRG1 is one of two catalytic subunits used by the SWI/SNF complex, the other catalytic subunit being hBRM.[4] The SWI/SNF complex is one of the major ATP hydrolysis-dependent chromatin remodeling complexes.[5] The complex has been observed to serve as an enzyme mechanism for

gene expression regulation. It has been demonstrated that SWI/SNF is involved in nucleosomal structure alteration, which exposes binding sites for transcription factors. Further studies have revealed that SWI/SNF participates in mechanisms that direct it toward targeted promoters which have the potential to either activate or repress transcription.[6] Notably, experiments have shown that, when separated from the SWI/SNF complex, BRG1 is capable of functioning alongside as few as one other SWI/SNF subunit to accomplish transcription activation as well as chromatin modification.[7]

The BRG1 subunit has been suspected to be capable of suppressing tumor formation. BRG1 has been found to function with BRM and interact with the protein product of the Retinoblastoma tumor suppressor gene product to repress the E2F transcription factor function.[8] Furthermore, a homozygous deletion of the carboxylic acid terminal region of BRG1 has been observed in prostate and lung carcinoma cell lines. Biallelic activations of BRG1 have also been discovered in several cancer cell lines including prostate, lung, breast, and pancreatic.[9] Tumor suppression ability has also been discovered with other subunits within the SWI/SNF complex, adding further legitimacy that the complex is connected with tumor suppression and regulation of cell growth.[10]

Although BRG1 has been found to act as a tumor suppressor, more recent research calls attention to its role as a tumor promoter. Many studies have proven that the expression of BRG1 is upregulated in cancers compared with healthy tissue.[11,12,13] BRG1's role as a tumor suppressor is, therefore, not its only role in cancer, and BRG1 has been discovered to be an oncogene.[14,15] In fact, BRG1 overexpression in cancers can be used as a prognostic indicator. With most cancers, including breast cancer, colorectal cancer (CRC), and prostate cancer, BRG1 upregulation is correlated with worse outcomes, underlining BRG1 as an oncogene.[12,16,17,18,19,20] However, patients with Non-small Cell Lung Cancer (NSCLC) who lacked BRG1 production had a worse prognosis than those with normal BRG1 expression, leading to divergent roles of BRG1 as tumor suppressor/promoter in different cancer types.[21]

While BRG1 treatments have been promising, a more comprehensive understanding of BRG1's effect on certain cancer pathways may allow for dual treatments consisting of BRG1 inhibition and upregulating/downregulating another pathway involved in cancer proliferation. Autophagy, apoptosis, PRMT5, and the RAS/RAF/ERK1/2 pathway are most significantly of interest to us. Analysis of autophagy and apoptosis with connection to BRG1 allows for an understanding of the mechanism behind BRG1's role in cancer formation. PRMT5 is a protein with significant effects on cancer growth and is highlighted in this review to emphasize the role BRG1 plays alongside it, whether cancer growth or inhibition. The RAS/RAF/ERK1/2 pathway is a signal transduction pathway with significant importance to researchers. *KRAS*, one of the three RAS genes, for example, is a necessary protein to study due to its frequent mutations in certain cancers.

This review will attempt to demonstrate the multifaceted effects BRG1 has on cancer sustainability and vitality. Treatment via knockdown of BRG1 is a growing trend for researchers of some cancers, and a synergistic approach via dual treatment may assist in cancer inhibition.

BRG1 Acts as a Cancer Promoter via Autophagy and Apoptosis Pathways

BRG1's potential role as an oncogene has prompted research in BRG1 drug and knockout treatments in several types of cancer. Knockdown of BRG1 in prostate cancer has been observed to have an inhibitory effect on tumor growth.[19] In CRC, the knockdown of BRG1 induces cell senescence[22] and apoptosis.[20] In triple-negative breast cancer, BRG1 knockdown has played a supporting role in tumor management in conjunction with chemotherapy treatment.[23] However, in NSCLC, knockout of BRG1 in lung cancer correlates with tumor progression, not suppression.[24] With the research of BRG1 targeted therapies becoming more promising, it is important to understand BRG1's role in cellular autophagy and apoptosis and the pathways involved.

Macroautophagy (referred to in this article as autophagy) should be of interest to researchers due to its nature as both a cancer proliferator and a cancer inhibitor. Autophagy is predominately triggered by nutrient starvation.[25,26] It is first initiated via the ULK1 complex, including proteins ULK1, ATG13, and FIP200. mTORC1 hyperphosphorylates ATG13, preventing autophagy in cells not undergoing starvation. The ULK1 complex initiates the VPS34/Beclin 1 kinase complex, containing VPS34, Beclin 1, VPS15, and ATG14L. This complex produces phospholipid PI3P, which binds with proteins to create the autophagosome. The ATG16L1–ATG5–ATG12 conjugation machinery and LC3 conjugated systems work with many ATG's to expand the autophagosome.[27,28]

Autophagy is generally considered a mechanism of tumor growth and metastasis because it is an attempt by the cell to maintain viability. Mutations of autophagy genes are quite rare in cancer,[29] which suggests that autophagy plays an important role in cancer development. Previous findings have found a mutation of the Beclin1 gene in breast, ovarian, and prostate cancer which provides evidence to support the hypothesis of autophagy functioning as a tumor suppressor.[28] However, others have disputed the explanation, since Beclin1 is adjacent to BRCA1, a tumor suppressor gene.[29] The debate over autophagy as a tumor suppressor or promoter is still unresolved. Therapies promoting and inhibiting autophagy are both being attempted.

Apoptosis is a cellular process initiating a programmed cell death, which consists of two main pathways: the extrinsic and intrinsic pathways. The intrinsic pathway is mediated by the mitochondria. This mediation can involve negative signals, such as a lack of growth factors, hormones, and cytokines, and positive signals, including an abundance of radiation, toxins,

hypoxia, hyperthermia, viral infections, or free radicals. The extrinsic pathway involves transmembrane death receptors.[30]

BRG1 has a prominent role in autophagy and apoptosis regulation. A comprehensive understanding of the proteins and pathways BRG1 utilizes will educate researchers on how BRG1 acts as a cancer proliferator, as well as what proteins are upregulated. This may allow for treatment methods designed to target the cell's ability to regenerate and induce cellular death.

BRG1 and Autophagy

The relationship between BRG1 and autophagy is complex, with a lack of investigation into the topic. Even less information has been discovered about the direct interactions between BRG1 and autophagic proteins. However, one study of intestinal epithelial cells did share findings of a meaningful connection between BRG1 and autophagy proteins. Cells lacking proper BRG1 expression had significantly reduced autophagy. This study also found a direct correlation between the deletion of BRG1 and a decrease in key autophagy regulatory genes, mainly ATG16L1, ATG7, Ambra1, and WIPI2.[31]

ATG16L1 and ATG7 are direct components of the ATG16L1–ATG5–ATG12 conjugation system. These proteins assist in LC3 phosphatidylethanolamine conjugation and autophagosome formation.[31] Ambra1 stabilizes the VPS34/Beclin 1 complex by binding to Beclin1, assisting in the formation of autophagosomes. It is inferred that Ambra1 is necessary for autophagy, as Ambra1 deficiency is correlated with impaired autophagy.[32] WIPI2 is one of four WIPI proteins which comprise PROPPIN (β-propellers that bind polyphosphoinositides). WIPI binds to PI3P as a method of signaling autophagic molecules. WIPI also assists in the lipidation of LC3 by recruiting and binding to the ATG16L1–ATG5–ATG12 complex.[33]

BRG1 has been documented to interact with p53.[22,31,34] p53 is a regulatory protein involved with both autophagy and apoptosis. In CRC, BRG1 has been shown to bind to SIRT-1 and enhance SIRT1-mediated deacetylation of p53 at K382. The knockout of BRG1 correlates with less efficient SIRT-1 deacetylation and increased stability in p53.[22] Another study of CRC found that BRG1 knockdown led to increased p53 expression and found evidence of a BRG1/CHD4/HDAC1 complex regulating p53 transcription and stability.[34]

Retinoblastoma protein (RB) and BRG1 also interact to initiate autophagy. RB is a cancer suppressor protein, initiating cell cycle arrest at the G1 checkpoint.[35] Together with BRG1, RB suppresses E2F1 activation functions.[36] E2F1 acts as an antagonist to RB-mediated autophagy. Therefore, the BRG1/RB suppression of E2F1 initiates autophagy.[37] Furthermore, BRG1 has been found to enhance RB inhibition of E2F and cyclin A.[38] This suggests that BRG1 may cooperate further with RB for autophagy initiation.

Contrary to studies supporting BRG1 as an autophagy inducer, some studies have reported that BRG1 functions as an autophagy inhibitor. A study of the hearts of mice found that mice with double mutant BRG1 & BRM showed an increase in mitochondrial autophagy (mitophagy).[39] This study differs in that BRM is mutated alongside BRG1. Therefore, there may be differences in autophagic flux when both SWI/SNF ATPase subunits are affected as opposed to just BRG1. A different study of renal fibrosis in vivo and in vitro found that overexpression of BRG1 inhibited autophagy and suggested this is accomplished through activation of the Wnt/β-catenin signaling pathway.[40] Further study of this pathway in varying cancer types in the context of BRG1 and autophagy is necessary for clarification of BRG1's role in autophagy.

Most research suggests BRG1 has a role in supporting autophagy at different stages of autophagy. However, more research into BRG1's effect on various cancer types is necessary to establish conclusive interactions and methods of treatment. The interactions BRG1 has with autophagic proteins are depicted in Figure 1.

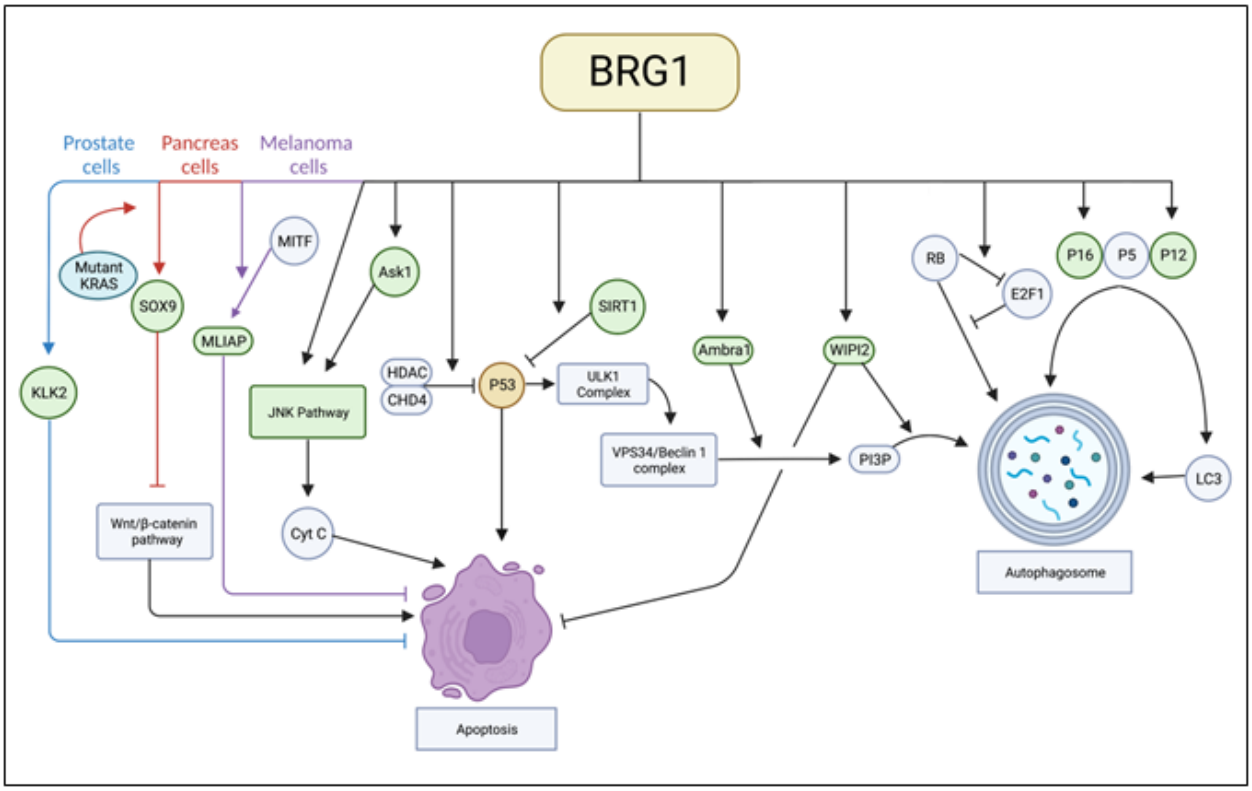

Figure 1. *A complete image of interactions between BRG1 and apoptotic and autophagic proteins.*

BRG1 and Apoptosis

BRG1's relationship with apoptosis has been documented with predominantly consistent results. Many studies have found that BRG1 expression is correlated with reduced apoptosis, and a

BRG1 deficiency increases apoptosis.[20,31,34,41,42] However, other studies have concluded that the loss of BRG1 in CRC and lung tumors had no significant effect on apoptosis.[22,43] The focus of these studies, however, was not BRG1's role on apoptosis, and each only performed one experiment documenting these results. Therefore, it appears most likely that BRG1 plays a role in apoptosis inhibition.

A study of BRG1 in CRC found that BRG1 suppression leads to upregulation in the JNK pathway.[44] The JNK pathway is a MAPK pathway that transduces extracellular signals. JNK is traditionally considered an apoptosis inducer through the intrinsic pathway by cytochrome c and caspase 3 activation.[45] A study of neuronal crest cells, which identified BRG1 as a transcriptional activator of PlexinA2, also found that BRG1 suppresses Ask1 and P21.[46] Ask1 (apoptosis signal-regulating kinase 1) is a MAPKKK protein that induces apoptosis mainly through the JNK pathway.[47] The method by which BRG1 suppresses Ask1 is not complete, and it is not specified if this is a cell-specific interaction.

Several studies have noted cancer-specific BRG1 apoptosis pathways. A study of melanoma cells exposed to UV light found that BRG1 inhibited apoptosis alongside MITF to permit transcription of the melanoma inhibitor of apoptosis promoter ML-IAP.[48] Additionally, a study of prostate cancer cells with inhibited BRG1 found that several of BRG1's target genes were decreased, including protein KLK2,[19] a known apoptosis inhibitor in prostate cancer.[49] Furthermore, a study of pancreatic ductal adenocarcinomas (PDAs) derived from intraepithelial neoplasia (panIN) in KRAS mutant cells found that BRG1 assisted in the initiation of panIN and its progression to pancreatic carcinoma.[50] BRG1 binds to the SOX9 promoter and helps initiate SOX9 transcription. SOX9 has been shown to have an effect on cancer proliferation, and SOX9 knockdown has a proapoptotic effect.[51] The inhibition of BRG1 has been observed to prevent panIN formation and prevent panIN-derived PDA through apoptosis induction. While the mechanism of BRG1's inhibition of panIN apoptosis is not investigated further, SOX 9 may be another factor due to its inhibition of apoptosis through the Wnt/β-catenin signaling pathway.

p53 is an apoptotic protein, discussed previously regarding autophagy, regulated by BRG1. p53 is known to induce apoptosis in early-stage tumor cells and cells with defective autophagy.[52] Another autophagic protein regulated by BRG1 that is involved in apoptosis inhibition is WIPI2. In a study of hepatocellular carcinoma (HCC) cells, the deletion of WIPI2 promoted apoptosis.[53] By upregulating WIPI2, BRG1 has another pathway to inhibit apoptosis.

BRG1's role as an apoptosis inhibitor has general and cancer-specific components. While the literature is not unanimous that BRG1 and apoptosis have a connection, treatment including BRG1 inhibition along with apoptosis induction may reap some unforeseen benefits. The interactions BRG1 has with apoptotic proteins are depicted in Figure 1.

BRG1's Interactions with PRMT5 in Cancer Development

Protein arginine methyltransferases (PRMTs) participate in several cellular pathways used for cell maintenance. Numerous studies have found an upregulation of PRMTs in cancer cells.[54] In particular, histone methylation involving Protein Arginine Methyltransferase 5 (PRMT5) is thought to repress transcription and has been observed to interact with several gene repression complexes[55] including some chromatin remodeling complexes which are dependent on ATP hydrolysis.[56] Notably, PRMT5 has been shown to interact with the SWI/SNF complex, as well as BRG1 specifically.[57] Together, they can perform methylation on the N terminus tails of the H3 and H4 histones.[58] Methylation of histone tails, such as H3 has been observed to have significant effects on transcription.[59] Also, it has been experimentally demonstrated that the tumor suppressor genes suppressor of tumorigenicity 7 (ST7) and nonmetastatic 23 (NM23) are both repressed because of histone modification by PRMT5 when in association with BRG1 and hBRM.[60] Furthermore, PRMT5 has been shown to induce autophagy and inhibit apoptosis in certain cancers.[61,62]

Several cellular processes are used to tightly coil DNA around nucleosomes and densely pack them together into histone packages for condensed storage. The core histones H2A, H2B, H3, and H4, form octamers each containing 145–147 base pairs of DNA.[63] Histone-modifying enzyme complexes work alongside ATP hydrolysis-dependent chromatin remodeling complexes to regulate transcriptional processes.[3] Histone modification can transpire through multiple mechanisms, including methylation, acetylation, phosphorylation, ubiquitination, and ADP-ribosylation. These processes affect the tail ends of histone proteins after the translation process and are suspected to influence chromatin structure. Lysine and arginine residues of H3 and H4 histones are primarily edited through methylation, although the enzyme mechanism is not fully understood.[64] As aforementioned, PRMT5 has been observed to function in histone methylation alongside SWI/SNF and BRG1, and it is therefore worth investigating their association.[57] Additionally, it is notable that deacetylation of histone H4 through Histone deacetylase 3 (HDAC 3) has been observed to significantly affect the silencing of apoptotic genes.[65]

BRG1 has been observed to function as both a tumor promoter and suppressor in association with specific protein complexes prior to oncogenesis. Often involved in those complexes is PRMT5 which contributes a histone methylation component. Histone methylation is a versatile cellular function which affects interaction between BRG1 component complexes and target genes which are associated with tumor regulation.

BRG1 Potentiates Cancer Development in Association with PRMT5 via H3 and H4 Histone Proteins

Numerous experiments have confirmed that methylation of histone 3 arginine 8 (H3R8) and histone 4 arginine 3 (H4R3) through PRMT5 interaction represses the transcriptional abilities of

the genes targeted. Furthermore, it has been observed in cellular differentiation studies that methylation of histones through PRMT5 also has a role in transcriptional activation.[57]

A study of prostate cancer provides evidence that PRMT5 is involved with the activation of androgen receptor (AR) transcription. PRMT5 binds to the AR gene's proximal promoter region and is involved with symmetric dimethylation of local H4R3 within the region. A high quantity of methylated H4R3 present indicates a high expression of PRMT5.[66] The Sp1 transcription factor for the AR interacts with PRMT5 to induce interaction between PRMT5 and the AR. In correlation with that process, a complex is formed between PRMT5 and BRG1, which is located on the AR gene's proximal promoter region. Knockdown of Sp1 induces downregulation of both PRMT5 and BRG1 from the proximal promoter region.[67] Thus, it can be inferred from this study that the activation of AR transcription, a component of prostate oncogenesis, is reliant on a complex composed of Sp1, BRG1, and PRMT5. Figure 2 depicts the association of PRMT5 and BRG1 in modifying the AR gene promoter structure.

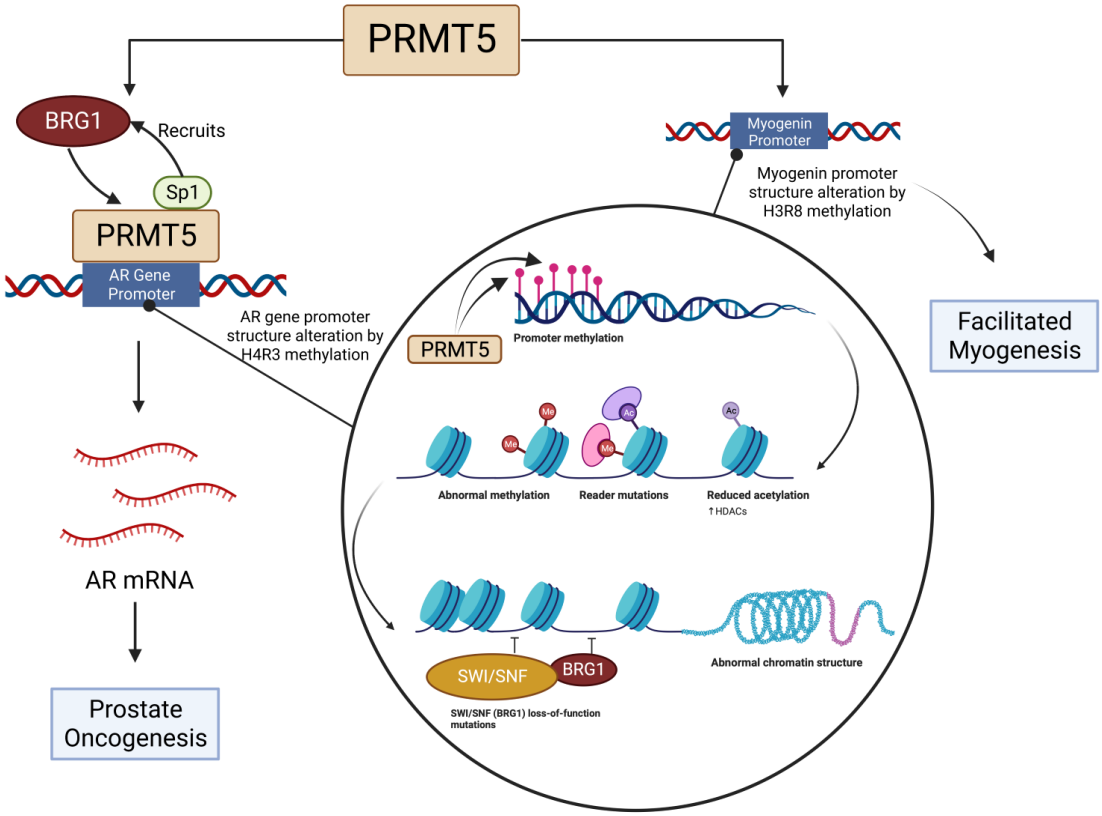

Figure 2. *BRG1 is recruited by Sp1 to bind with PRMT5 at the auxiliary AR gene promoter region, which is altered as a result of H4R3 methylation, initiating prostate oncogenesis. The myogenin promoter is altered and structure changed by PRMT5 and BRG1 association, leading to facilitated myogenesis.*

Certain interactions between PRMT5 and BRG1 have been theorized to be a necessary component for BRG1's function. Without the aid of a histone-modifying enzyme, such as PRMT5, ATP-dependent chromatin remodeling enzymes, such as BRG1, will not function. Relationships between BRG1 and other proteins in the SWI/SNF complex in relation to myogenesis have been extensively studied.[68] Similarly, PRMT5 has been connected to the modification of histones, in particular H3 and H4, which impact gene regulation during the process of skeletal muscle differentiation.[69] It has been discovered that BRG1 is responsible for changing the promoter structure of myogenin in association with dimethylated H3R8 and PRMT5. Without BRG1, differentiation was downregulated. Similarly, the removal of PRMT5 also diminished differentiation levels.[70] Figure 2 depicts the association of PRMT5 and BRG1 in modifying the myogenin promoter structure.

Furthermore, overexpression of PRMT5 associated with BRG1 was found in association with an upregulation of the cell cycle regulators: Cyclin E2, Cyclin B2, and CDK4.[60] Upregulation of these regulators has been observed in several tumor types.[71,72]

PRMT5's histone methylation function, which is linked to transcriptional activation, is found to be responsible for oncogenesis. In certain cancers, BRG1 has been observed to correspond with PRMT5 in that process. Intervention within that complex may produce favorable results in oncogenetic tumor prevention.

BRG1 Suppresses Tumor Development and Regulates Transcription of Myc/Max/Mad Genes in Connection with PRMT5

It has been observed that downregulation of the tumor suppressor genes suppressor of tumorigenicity 7 (*ST7*) and nonmetastatic 23 (*NM23*) have been directly correlated with a downregulation of PRMT5. Such downregulation was determined to affect PRMT5-associated BGR1 complexes which performed methylation of H3R8. Direct crosstalk between BRG1 and *ST7* was discovered, but not with *NM23*. However, hBRM was discovered to be directly associated with *NM23* but not *ST7*. Interestingly, both BRG1 and hBRM interact directly with *MYT1l*, suggesting the SWI/SNF complex to be a knockout target for intellectual disability.[60,73] Furthermore, it was determined that even without overexpression of PRMT5, there is an association between BRG1 and PRMT5.[60] Transcription levels of NM23 specifically were observed to decrease while displaying nucleoside diphosphate kinase activity, indicating high levels of metastasis within the cells overexpressing PRMT5.[74] Within the SWI/SWF complex, BRG1 has the ability to modify the accessibility of DNase and restriction enzymes on mononucleosomes.[75]

It is important to examine the complex formed between histone deacetylase 2 (HDAC2) and the mSin3A corepressor, which functions in chromatin remodeling, and its interaction with BRG1 and the SWI/SNF complex. PRMT5 is also added to the complex in the effect of notably

interacting with BRG1, hBRM, and BAF45/Ini1.[76] Histone deacetylation of K9 on H3 and H4 has been observed to enhance their methylation by PRMT5-associated SWI/SWF complexes.[77] Additionally, studies have determined that Mad-Max heterodimers suppress transcription in association with mSin3A and HDAC2.[78] Thus, a correlation between BRG1 and this complex is of note in the chromatin remodeling process, which may play a role in Myc/Max/Mad gene repression.[58]

Furthermore, another study has tested a possible linkage between said transcription repression mechanism and BRG1 and hBRM with the theory that they may take part in the suppression of Myc/Max/Mad genes, which are possible targets to regulate cell proliferation.[58,79] BRG1 has also been observed to have a role in c-Myc interaction with BAF45/Ini1 and transcription activation.[80] In one study, it was hypothesized that BRG1 may repress the function of target genes, thus making the *cad* promoter region a site of interest. A knockdown of BRG1 resulted in *cad* repression which supported the hypothesis that it is a component supporting *cad* function, indicating a role of histone methylation and chromatin remodeling. Figure 3 depicts the association of PRMT5 and BRG1 in the mSin3a/HDAC2 complex. However, not all Myc/Max/Mad target genes are affected by BRG1. It was observed that *nuc* mRNA levels which were depressed by histone deacetylation through depsipeptide treatment were not even furthermore depressed within the introduction of BRG1.[58] It can be concluded that the magnitude of repression due to BRG1 and Myc/Max/Mad interaction is dependent on the target gene.

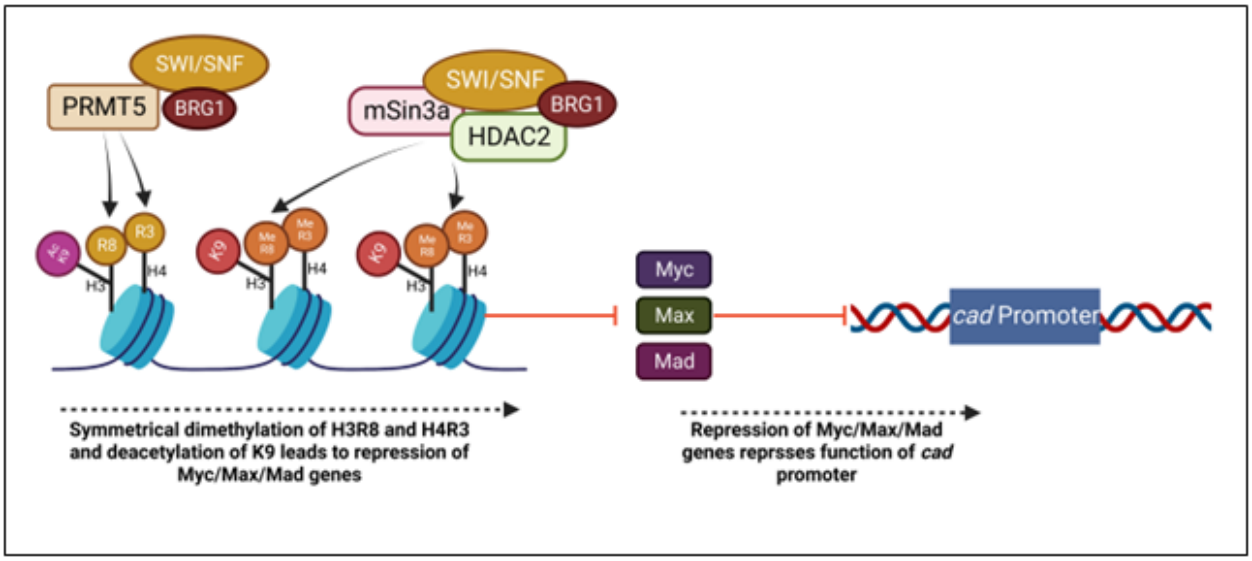

Figure 3. *Histones H3R8 and H4R3 are symmetrically dimethylated, while K9 is deacetylated through PRMT5 and BRG1 association. A complex of PRMT5, BRG1, mSin3a, and HDAC2 repress Myc/Max/Mad genes, leading to additional suppression of cad promoter.*

BRG1 association with PRMT5 has been demonstrated to function as a tumor suppressor in correspondence with certain target genes. Further investigation into the binding complex and complementary nature of BRG1 and tumor suppressive complexes in the mechanisms discussed and others would be beneficial to the development of tumor regulating treatments.

BRG1 and Oncogenic RAS/RAF Pathway

Rat sarcoma virus (RAS) proteins are a family of GTPases involved in signal transduction, especially in the Ras/Raf pathway. There are three main RAS proteins, H-Ras, N-Ras, and K-Ras (referred to as KRAS in this article). KRAS is most significant to us because it is often mutated to be overexpressed in a number of cancers, leading to excessive cancer proliferation. Activating mutations are most common in pancreatic, thyroid, colorectal, and lung cancers (95–35%).[81]

Mitogen-activated protein kinases (MAPKs) are signaling pathways that regulate cellular growth, stress responses, differentiation, and viability.[82,83] Four cascades have been identified within MAPKs, including extracellular signal-regulated kinases 1 and 2 (ERK1/2), c-Jun N-terminal kinase (JNK), p38, and ERK5. ERK's 1 and 2 (ERK1/2) are essential for regulating cell signaling and play a role in tumorigenesis.[84] ERK1/2 is stimulated in response to the sequential phosphorylation of Ras/Raf proteins, including several MAP3Ks and MAPK/ERK kinases (MEK).[85] The Ras/Raf/MAPK/ERK1/2 pathway is one of the most prolific signal transduction pathways for tumorigenesis.[84,86] MAPK cascade signals enter the nucleus and have been proven to modulate post-transcriptional genes through interaction with transcription factors and chromatin remodeling enzymes, such as SWI/SNF.[87] Thus, an analysis of BRG1's interaction with other proteins of the ERK1/2 pathway would be beneficial to prescribe treatment for the pathway. It is also noteworthy, considering PRMT5's interaction with BRG1, that knockdown of PRMT5 interaction with the Ras/Raf and MEK proteins in correspondence with the ERK1/2 pathway has been exhibited to directly downregulate ERK1/2 in combination therapies.[88]

BRG1 and Oncogenic KRAS in Cancer Formation

Previous research has discovered that BRG1 is an important protein involved in oncogenic KRAS-induced tumor growth of acinar and lung cells. However, depending on the origin of cancer development, BRG1 may act as either a suppressor or promoter.

Oncogenic KRAS is a necessary mutation for the formation of pancreatic ductal adenocarcinoma (PDAs). A total of 90% of human PDA samples have a KRAS mutation.[89] BRG1 serves a dual role in this process as a KRAS supporter and KRAS antagonist. Pancreatic ductal adenocarcinoma may be formed via PANIN, MCN, and IPMN. BRG1 has been shown to block IMPN formation and prevent oncogenic KRAS-driven PDA formation from IPMN.[90] Further study, however, found that BRG1 regulates SOX9 transcription and supports oncogenic KRAS-induced PANIN formation as well as oncogenic KRAS-induced PDA derived from PANIN.[50] Together, these studies support BRG1 as a key protein within oncogenic

KRAS-initiated pancreatic cancer. Furthermore, these studies highlight the dual role of BRG1 as a promoter or suppressor of cancer, even within the same cancer of varying origin.

Research in NSCLC has supported BRG1 as an instigator of KRAS-induced cancer. KRAS mutant tumors with active BRG1 were more proliferative than KRAS mutants without BRG1 expression.[43] Another study found that BRG1 inactivation alone did not promote tumor progression. Although, the inactivation of BRG1 and P53 as well as the activation of oncogenic KRAS resulted in highly penetrant lung adenocarcinomas compared to the inactivation of just p53 and activation of oncogenic KRAS. Furthermore, when treated with an oxidative phosphorylation inhibitor, only the growth of cells with BRG1 inactivation was inhibited.[91] Therefore, BRG1 has a role in protecting KRAS cancers.

Patients with NSCLC which present a mutation that inhibits BRG1 expression had increased mutations in the KRAS gene compared to those without BRG1 mutations.[92] Conversely, KRAS mutant NSCLC patients were more likely to have intact BRG1 than those with KRAS wildtype.[43] Furthermore, NSCLC patients with both KRAS and BRG1 mutations had worse survival outcomes in immunotherapy and non-immunotherapy treatments.[93] The increased rates of KRAS mutations in BRG1 mutant patients as well as the worse outcomes for patients with co-mutations may suggest a role for BRG1 in reducing the effects of oncogenic KRAS and cancer formation.

Oncogenic RAS-induced senescence has been shown to regulate BRG1/BRCA1 interactions, and RAS knockdown increased BRG1 expression. Furthermore, BRG1's association with promoters CDKN2a and CDKN1A to express p16 and p21 is upregulated during RAS knockdown-induced senescence.[94]

Further BRG1/KRAS interaction in other cancers may support a greater understanding of how to properly treat KRAS mutated cancers.

BRG1 and ERK/MAPK

It has been observed that BRG1 is inactivated as a result of ERK1/2 phosphorylation. However, BRG1 reactivation was observed upon the introduction of general protein phosphatase inhibitors.[95] A study examined the regulation of a complex comprising BRG1 and the heat shock factor 4b (Hsf4b) protein in response to ERK1/2 activation and inactivation.[96] Heat shock factors are generally involved with acute stress regulators through the regulation of the transcription of genes responsible for stress protein and molecular chaperone production, such as heat shock protein 70 (HSP70).[96,97] However, alterations in the roles of heat shock factors have been observed through the malignant transformation of cells.[98] Notably, Hsf4 involves itself in olfactory neurogenesis and has been noted to be overexpressed in liver and colon cancer.[99,100,101] A previous study in which HeLa cells were co-transfected with Hsf4b as well as active MEK resulted in phosphorylation and an increase of ERK1/2 activity.[102] Experiments confirmed these

findings and additionally discovered a greater association between slow-migrating BRG1 and Hsf4b. Additional experiments in HeLa cells transfected with Hsf4b and combinations of MEK and dual specificity phosphatase 26 (DUSP26) produced more significant results.[96] DUSP26 has been observed to be a tumor suppressor and an oncogene in different cellular contexts and is able to inactivate MAPK in vivo.[103] Experiments revealed that an association between BRG1 and Hsf4b is indeed upregulated by the expression of MEK. Similarly, a lack of MEK or a presence of both MEK and DUSP26 downregulated the correspondence between BRG1 and Hsf4b. It can thus be concluded that activation of MAPK and ERK1/2 promotes BRG1 and Hsf4b association. Such association was determined to be cell cycle-dependent and alters Hsf4b's ability to bind to DNA, resulting in the negative effects noted above.[96,103] Further research into the binding of BRG1 and Hsf4b through the activated ERK1/2 pathway would be beneficial in inhibiting the complex. DUSP26 has presented itself to be a promising inhibitor, and its interactions with individual components of the ERK1/2 pathway should be studied more.

BRG1 and the ERK1/2 pathway exhibit similar notable responses to treatment. In vascular smooth muscle cells (VSMC), the introduction of BRG1 overexpression proved to increase the amount of protein expression of proliferating cell nuclear antigen (Pcna), an assisting protein to DNA polymerase in several malignant cell types, and platelet-derived growth factor (Pdgfα).[104,105] As a result, VSMC proliferation increased. In a BRG1-shRNA group, where BRG1 expression was silenced, the amount of Pcna, Pdgfα, as well as neurotrophin-3 (Ntf3), were all reduced. Hydrosulfuric acid presented itself as an effective inhibitor of the three proteins. Phosphorylation of ERK1/2 displayed a similar trend in its activity as BRG1, Pcna, Pdgfα, and Ntf3. The results of this study indicated that hydrosulfuric acid is an effective inhibitor of VSMC proliferation through the MAPK pathway when there is a downregulation of BRG1.[106]

Studies have suggested that casein kinase 2 (CKs) is a mediator of BRG1 phosphorylation. However, the effect of CK2 phosphorylation of BRG1 is only speculatory. One study indicated that it modified the organization of telomeres as well as debilitating topologically associating domain (TAD) boundaries.[107] Another study asserted that it involves BRG1 with the nuclear structure and overall genomic organization.[108] Hyperphosphorylation of BRG1 by CK2 in mitosis is not the only source of its phosphorylation, as noted earlier in this section. MAPK also serves as a phosphorylation mediator of BRG1 in mitosis, thus acting as a controller of its chromatin remodeling function. It is inferred that the sites of phosphorylation by CK2 and MAPK are different and thus may not be the only acting kinases on BRG1.[109] Further investigation into the existence of such other kinases and whether their association with BRG1 is similar or different would be beneficial. Additionally, besides determining if such kinases act independently, the possibility of these kinases acting in conjunction with each other should be explored.

There is significant evidence that BRG1 interacts with numerous components of the Ras/Raf/MAPK/ERK1/2 pathway. Furthermore, BRG1 has been noted to respond to treatment in concert with the ERK1/2 cascade. Activation of the ERK1/2 cascade has also presented itself to promote BRG1 interaction with other proteins. Associations between BRG1 and other proteins in correlation with cell malignancy beyond those noted in this article should be explored further.

Conclusions

In conclusion, our review emphasizes the importance of research into BRG1-related pathways in many cancers, as well as the possibility of success of BRG1 knockdown in conjunction with another treatment. Much of the evidence suggests BRG1 supports autophagy and inhibits apoptosis. These are two methods by which BRG1 supports cancer growth and prevents treatment. BRG1 works with cancer-specific proteins to inhibit apoptosis, and the knockdown of BRG1 may support other treatments such as chemotherapy. PRMT5 has demonstrated itself to both bind and associate with BRG1 to accomplish histone methylation. Through such methods, BRG1 is able to function within complexes to regulate both gene activation and repression. Notably, several of those complexes regulate tumor suppressor genes. Research into protein binding within such complexes may be useful to specify targets for treatments to either enhance or diminish BRG1's association depending on the target gene as a preventative method. BRG1 has displayed a role in oncogenic KRAS-induced cancers with directionality seemingly dependent on cancer type. BRG1 associates with several proteins within the Ras/Raf/MAPK/ERK1/2 pathway and is promoted to form complexes with other proteins when ERK1/2 is activated. Given that BRG1 and PRMT5 both play a similar role in apoptosis and autophagy and are both involved in the RAS/RAF pathway, further insight into the effects of dual knockdown may show promising results. This paper hopes to emphasize the possibility of cancer treatments benefiting from the co-treatment of BRG1.

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*This paper has been previously published in the International Journal of Molecular Sciences February 2023.

Yeshiva University Journal of Medicine and Dentistry

Review

COVID-19 and the Immune Response: A Multi-phasic Approach to the Treatment of COVID-19

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Abstract

COVID-19 is a viral agent causing flu-like symptoms that, when exacerbated, can have life-threatening consequences. COVID-19 has also been linked to persistent symptoms, sequelae, and medical complications that can last months after the initial infection. This systematic review aims to elucidate the innate and adaptive immune mechanisms involved, identify potential characteristics of COVID-19 pathology that may increase symptom duration, and describe the different stages of COVID-19 infection as well as each phase's corresponding immune response. Thus, by approaching COVID-19 infection with a multi-phasic perspective, we encourage the employment of different treatments at specific stages, thereby fully curating the treatment to the stage of disease.

Introduction

Coronavirus disease 2019 (COVID-19), a viral infectious disease, is caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] As of writing, there have been an estimated 500 million cases of COVID-19 and more than 6 million deaths.[2] Most patients with COVID-19 experience minimal flu-like symptoms, or even none at all. However, approximately one fifth of COVID-19 patients do experience severe disease or die.[3] Underlying medical conditions and advanced age are known to be risk factors for severe COVID-19.[4]

SARS-CoV-2 is similar to other coronaviruses in that it is an enveloped, spherical virus with a single-stranded, positive-sense RNA genome. Therefore, the entire viral life cycle takes place within the host cell which has been infected. The virus enters the host cell by using its viral spike (S) glycoprotein to interact with angiotensin-converting enzyme 2 (ACE2), a host receptor protein.[1] Transmembrane serine protease 2 (TMPRSS2), the second protein involved in this entry process, facilitates the fusion of viral and host membranes, and allows the virus to be released into the host cytoplasm. As such, viral tropism of SARS-CoV-2 is determined by the presence of ACE2 and TMPRSS2 on the host cell's plasma membrane, meaning that tissues like nasal epithelial cells, lungs, and bronchial branches—where co-expression of both is high—are affected most.[3,5,6]

The incubation period between SARS-CoV-2 exposure and symptom onset is about five days, although this may vary.[7] COVID-19 infection is known to be multi-phasic, meaning that there

are several stages to the infection process. This makes patients with severe COVID-19 infection difficult to treat, as different therapeutic approaches are required depending on the patient's stage of infection.[8] For example, the first phase of COVID-19 infection is a viral replication phase during which it is most beneficial to use drugs that inhibit viral replication. However, during the subsequent phase of inflammation, during which an overwhelming and potentially harmful immune response takes place, only drugs that reduce the immune response will be helpful.[9] For this reason, it is important to understand the immune system's full breadth of response to COVID-19 infection.

The first phase of infection involves a viral replication phase. In this phase, SARS-CoV-2 establishes itself in the host body and proceeds to rapidly duplicate itself. In the following two sections we describe the ways in which the innate and adaptive immune responses behave during this stage. We delineate the ways the virus escapes immunity and describe how the initial immune response brings about the second stage of infection, that of immune hyperactivation. The fourth section of this paper looks at that second phase alongside viral clearance, while the fifth section explores a phenom known as Post-Acute Sequelae of COVID-19—a proposed third stage of COVID-19 infection. Finally, the sixth section of our review details current treatment practices for COVID-19.

Innate Immune Response

Immune Detection of SARS-CoV-2

In order to initiate the immune response, immune cells have several types of pattern recognition receptors (PRRs) which recognize broad characteristics of pathogens. These are called pathogen-associated molecular patterns (PAMPs), or damage-associated molecular patterns (DAMPs). Many PRRs have been shown to activate in response to SARS-CoV-2.

Both the absence of, and interference with, toll-like receptor 2 (TLR2) has been shown to decrease pro-inflammatory response to SARS-CoV-2, suggesting that TLR2 is one of the PRRs that recognize the virus. Specifically, inhibition of TLR2 decreased inflammatory response after it had been induced with SARS-CoV-2 envelope protein (E protein), suggesting that E protein may be that which TLR2 recognizes on SARS-CoV-2.[10] Other TLRs are less closely studied in the context of SARS-CoV-2. TLR3, which recognizes double-stranded RNA (dsRNA), has been shown to be activated by SARS-CoV-2 infection within the first 24 hours.[11] TLR1, TLR4, and TLR6 may bind SARS-CoV-2 spike protein.[12] However, S protein has been shown to preferentially bind lipopolysaccharide, a known target of TLR4, casting some doubt over these findings, considering the possibility of lipopolysaccharide contamination of S protein causing a TLR4-mediated cytokine reaction.[13] TLR7 has also been shown to activate in response to SARS-CoV-2, and abnormalities in the *TLR7* gene correlate with severe COVID-19.[11,14]

SARS-CoV-2 Avoids Innate Immunity

SARS-CoV-2, in its interest of successfully infecting, has strategies to avoid being detected or acted upon by the immune system. Several studies have together shown that the SARS-CoV-2 immune evasion strategy involves restricting the interferon (IFN) system, resulting in low type I and II IFN responses, as well as low IFN-stimulated genes (ISGs) during the early stages of COVID-19.[15,16] The SARS-CoV-2 proteins responsible for this include non-structural protein 1 (NSP1), NSP8, NSP9, NSP13, NSP15, ORF9b, and ORF6.[17,18,19] This provides for SARS-CoV-2 to establish itself in the body without as much a threat from early immune response.

Complement Activation

The body's most immediate line of defense, the complement system, is usually vital for quick and effective immune response, but in coronaviruses may sometimes cause more harm than good. While complement activation during the first week after infection can successfully fight COVID-19, prolonged complement activation can lead to a positive feedback loop in inflammation that contributes to multi-organ failure in severe cases of COVID-19.[20] In mouse studies of the closely related SARS-CoV, it was found that products of C3 activation such as C3a, C3b, and iC3b were detectable in the lungs a single day after infection, and that $C3^{-/-}$ mice had less severe lung injury, had fewer neutrophils and inflammatory monocytes, and had reduced cytokine and chemokine levels. Additionally, $C3^{-/-}$, factor $B^{-/-}$, and $C4^{-/-}$ mice lost less weight over the course of infection than wild type mice.[21] Furthermore, experiments blocking C3 or C5 have been found to reduce disease severity, respiratory impairment, and cytokine response.[22]

Recently, it has also been reported that SARS-CoV-2 nucleoprotein (N protein) dimers activate mannose-binding protein-associated serine protease 2 (MASP-2), which is the main trigger for activation of the lectin pathway of the complement system. This in turn yields C3 convertase and the membrane attack complex (MAC).[23] Conversely, suppressing either the N protein-MASP-2 interaction or complement activation led to less lung injury.[23] SARS-CoV-2 thus stimulates the complement system, resulting in extended complement activation and inflammation.

These findings are further substantiated. For example, patients with macular degeneration, a complement-mediated disease, were found to have a much higher risk of developing severe COVID-19, suggesting a possible connection between the increased presence of complement proteins and worse COVID-19 outcome.[24] Additionally, patients with COVID-19 are found to have raised levels of complement proteins in their plasma and complement fragment deposition in certain organs.[25,26] They are also found to experience neutrophilia, the over-abundance of neutrophils in the blood, to the extent that the neutrophil:leukocyte ratio has been shown to be an independent risk factor for serious COVID-19.[27,28] Activated neutrophils and neutrophil extracellular traps (NETs) contain complement proteins necessary for the alternative C3 convertase, providing yet another way for COVID-19 to induce prolonged complement activation.[20,29]

IFN and IL Response

Similar to what has been suggested in the complement system, the induced innate immune response to COVID-19 is both necessary for effective disease suppression, as in other diseases, and capable of causing severe damage to the host. In their intended role, IFNs help clear infection from the host body by promoting the production of antiviral compounds by transcription of ISGs and cytokines. However, in severe COVID-19, positive feedback loops in cytokines and IFNs can lead to cytokine storm—the dysregulated release of cytokines—leading to hyperinflammation, multiorgan failure, and death.

Upon detection by PRRs, immune cells such as macrophages (MQs), dendritic cells (DCs), and natural killer cells (NK cells) release IFNs and proinflammatory cytokines including IL-1□, IL-6, TNF-α, IL-12, and IFN-γ.[30] TNF-α and IFN-γ together stimulate PANoptosis—an innate immune programmed cell death pathway separate from apoptosis, pyroptosis (inflammatory programmed cell death), or necrosis (programmed cell death by necrosis)—which in turn stimulates further proinflammatory cytokine release, resulting in cytokine storm (Figure 1).[31] The prolonged inflammation and associated endothelial cell damage can eventually contribute to symptoms of severe COVID-19 such as lung damage, acute respiratory distress syndrome (ARDS), organ failure, or even death.[32] This pathway is especially seen in the second week after disease onset, following the decline in IFN seen in the earliest phase of disease. Regulation of the IFN system is then of great importance to COVID-19 outcomes, seeing as how under activation in the early stages allows SARS-CoV-2 into the body without detection, and overactivation in later stages results in serious damage to the host.

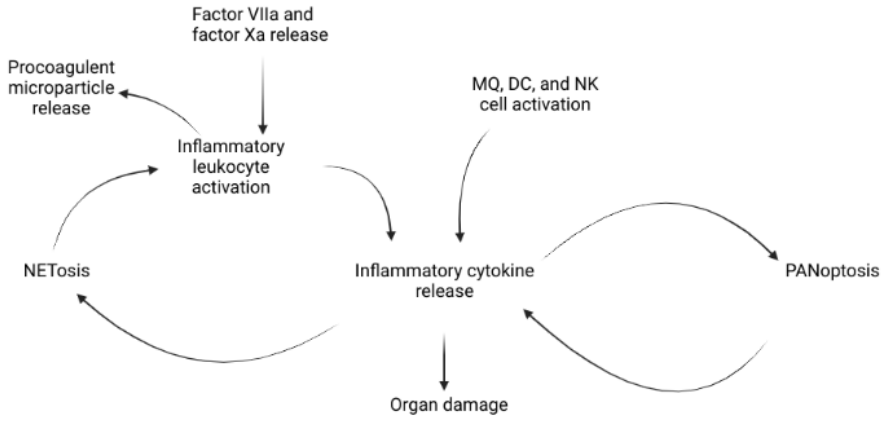

Figure 1. *Inflammatory cytokines are present in both cytokine storm and procoagulant feedback loops.*

These inflammatory responses can cause further damage by contributing to thromboinflammation, a coagulatory response to systemic inflammation through thrombin generation. On infection, monocytes and subendothelial cells release factor VIIa and factor Xa. Leukocytes, endothelial cells, and platelets release proinflammatory cytokines and procoagulant microparticles, promoting increased leukocyte adhesion and decreased vasculo-protective molecules. These result in NETosis, the activation and release of neutrophil extracellular traps (NETs), which recruit yet more inflammatory leukocytes and cytokines (Figure 1). Eventually this can lead to a loss of homeostasis and damaged microvasculature, called disseminated intravascular coagulation (DIC).[33] Thus, several positive feedback loops encourage the release of proinflammatory cytokines and can result in serious damage to the host.

Adaptive Immune Response During Covid

Adaptive Immune Response Time to COVID-19

The adaptive immune response takes at least five days to take effect.[7] For COVID-19 it was found that antibodies are detectable approximately six days after RT-PCR detection of viral infection.[34] The milder cases of COVID-19 generally have longer response times for showing detectable levels of antibodies, with some being up to 28 days post RT-PCR confirmation of viral infection.[34] The deadly cytokine storm that some severe cases of COVID-19 patients had was generally triggered about one week post infection.[34] Finally, it was found that antibodies last longer in patients who had severe cases of COVID-19, generally lasting at least six months, while more mild cases had antibody levels that faded by 2-4 months post viral clearance.[34]

Antibodies for COVID-19

Antibody responses to COVID-19 are essential for viral clearance. Antibody maturation increases the body's ability to defend against SARS-CoV-2 infections.[35,36,37] When tested several months after infection, sera was discovered to have low antibody levels specific for single variants of SARS-CoV-2, but high levels of antibodies capable of recognizing the common epitope of several variants.[35] Additionally, even more antibody variance was prompted by repeated exposure to slightly different variants of the coronavirus.[35]

Another study using plasma taken from 1-10 months post SARS-CoV-2 infection showed that initially the antibodies only protected well against the original variant that the patient was infected with, but plasma taken further from initial infection showed higher protection against variants of concern (VOCs). This indicates that although the total amount of antibody in sera may be declining, the protection offered against different variants of SARS-CoV-2 infection may not be declining much, if at all.[37]

However, another study showed that protection offered from vaccines against different variants of SARS-CoV-2 was not guaranteed.[38] This study found that a relatively small number of mutations could lead to an escape from immune neutralization. However, it is important to keep

in mind that this study was performed only a few weeks after vaccination, not giving the antibodies as much time to mature. Additionally, not all participants received the full schedule of vaccine doses that is recommended, and only half of the VOCs tested were even able to partially escape neutralization from the vaccine induced humoral immunity.[38] Table 1 summarizes the results of several studies which concluded that the protection against variants of concern following infection with SARS-CoV-2 is increased in the months following infection as antibodies mature, and antibodies that target more broad epitopes found on coronaviruses have favored propagation over specific epitope binding antibodies.

Table 1. Protection Against Variants of Concern (VOCs) of SARS-CoV-2.

Severity of infection	Time post infection	Protection Level against VOCs	Sources
Severe	1-3 months	Low to high	[35-37]
	4-6 months	Medium to high	
	6^{+} months	High	
Mild	1-3 months	Low to medium	[35-37]
	4-6 months	Medium to high	
	6^{+} months	Medium to high	

Finally, one more hopeful study showed that following receipt of a third dose of the BNT162b2 mRNA COVID-19 vaccine, the likelihood of a severe outcome from any of the variants of COVID-19 was highly reduced.[39]

$CD8^{\pm}$ & $CD4^{\pm}$ T-Cell Response for COVID-19

One study examined the effect prior COVID-19 infection has on $CD4^{+}$ and $CD8^{+}$ T-Cell responses to COVID-19 vaccination.[40] Regardless of infection status, upon vaccination, $CD4^{+}$ T-Cells immediately rose to higher levels. However, the cytotoxic T-cells only rose to high levels after a single dose for individuals that already had a SARS-CoV-2 infection, while those who were naive only had a boost in cytotoxic T-Cells following a second dose several weeks after the initial vaccination.[40] This provides evidence of strong memory T-cells and prolonged immunity even following the loss of serum antibodies for COVID-19.[41]

The importance of memory B and T cells in prolonged immunity to COVID was further shown in a study by Cox et al.[42] There, it was shown that, although antibodies produced in response to mild COVID-19 infection only have a 21-day half-life, the memory T and B cells formed maintain their protective capabilities from similar strains of SARS-CoV-2 for much longer. Research from similar human coronaviruses has shown that memory T and B cell responses last for several years after infection, and that memory T cells are essential in the quick response of B cells in antibody making and in the formation of cytotoxic T cells.[42]

Natural Killer Cells' Response to COVID-19

NK cells play a key role in the immune response to COVID-19. Natural killer cells work to attack and lyse infected body cells containing COVID-19 viral RNA and thereby allow the antigens into the bloodstream to be detected by other parts of the adaptive immune system. This forms a targeted response to the COVID-19 viral RNA, spike protein, and other antigens. In a recent study, the immune cell count that correlated the most with survival rate and least with severity of disease was the NK cell count.[43]

Immune Clearance of COVID-19

Immune Cells Through Infection

The efficiency of viral clearance for COVID-19 is significantly affected by $CD4^+$ and $CD8^+$ T cells. Virus-specific $CD8^+$ T cells have been associated with better outcomes in COVID-19 infections, as they kill infected cells with their cytotoxins. $CD8^+$ T cells are crucial for clearance of many viral infections.[44] COVID-19 virus clearance requires both adaptive and innate immune responses, but in innate immunity, macrophages can contribute to disease progression. A significant amount of the cytotoxin IL-6 is produced by macrophages during COVID-19, suggesting they may contribute to excessive inflammation.[45] The innate immune system dominates the early immune responses to viruses. Within this early response, many leukocytes are secreted including neutrophils, monocytes, plasmacytoid dendritic cells (pDCs), and natural killer (NK) cells. Once an adaptive immune response is triggered, T and B cells become critical for viral clearance which develops over days to weeks.[46]

Among the factors that may impede viral clearance of COVID-19 are decreases in the number of circulating NK cells, Th1 $CD4^+$ T cells, pDCs, phagocytic neutrophils and monocytes, as well as the immunomodulatory properties of progesterone, which is elevated in pregnancy. Factors that may exacerbate COVID-19 morbidity through hyperinflammatory states include increases in the complement system, increases in TLR-1 and TLR-7, and increased pro-inflammatory cytokines such as IL-6 and TNFα. Increases in complement system activity are linked to greater lung injury.[47] It has been well studied that during viral infections, a decrease in Th1 reactivity can result in less efficient clearance of infected cells. However, an overt Th1 and Th2 response to COVID-19 has been implicated in the pathogenesis of severe COVID-19.[48]

Early Infection vs. Late Infection

Another factor that remains unclear in viral clearance of COVID-19 is ACE2. ACE2 is a key component of the renin-angiotensin system, which cleaves angiotensin II to generate ang1-7. Increases in vascular permeability and immune cell infiltration is associated with lung edema due to angiotensin II accumulation in the lungs, and the reduction of ACE2 expression has contributed to acute lung failure through modulation of the renin-angiotensin system.[49] It has been reported that the expression levels of ACE2 played an important role in determining the

outcomes of COVID-19 infections. During the early stage, lower levels of ACE2 in the lung is beneficial for the host to control viral transmission and replication. However, it is possible that if not enough ACE2 is present for a prolonged period, the resulting lack of ACE2 could cause angiotensin II to be converted less effectively to ang1-7. Consequently, the accumulated angiotensin II might cause increased immune activity and eventually lung disease.[49]

In order to clear an infection effectively, patients must possess $CD8^+$ effector T cells that can kill virally infected cells, as well as $CD4^+$ T cells that can enhance the $CD8^+$ and B cell responses. However, cytokine release by T cells can also contribute to severe tissue inflammation and toxicity, resulting in mortality.[50] While cytokines are critical for the innate immune response and successful clearance of viral infections, their release must be controlled to prevent systemic cytokine storm and harmful inflammation during COVID-19 infection.[51] Therefore, immune checkpoints are significant because they help regulate effector T cell responses. If short term viral clearance is achieved, the majority of virus-specific T cells undergo apoptosis, but for long term viral clearance, the retention of the virus-specific memory T cell population is necessary.[50]

Factors Necessary for Viral Clearance

It has been shown that humoral immunity is not vital in clearing acute COVID-19 if there are sufficient amounts of $CD8^+$ T cells, and that the major role of $CD4^+$ T cells in the clearance of COVID-19 is to instruct humoral immunity with a much lighter role in amplifying cellular immunity.[52] In studies with B cell-deficient mice, antibodies alone were successful in clearing COVID-19, albeit slower than when paired with a fully competent adaptive immune response. However, viral clearance was impossible if neither $CD4^+$ nor $CD8^+$ T cells were present. In line with these findings, antigen-specific $CD4^+$ T cell profiling of acute and convalescent COVID-19 patients indicated that circulating T follicular helper cells play a role in reduced disease severity, further proving that antibody promotion of $CD4^+$ clearance is important.[52] Moreover, studies on COVID-19 patients showed that antigen-specific $CD4^+$ T cells could be detected as early as 2 to 4 days following symptom onset, and this early detection was associated with improved outcomes. It appears that both humoral and cellular immunity contribute to COVID-19 clearance during primary infection, which is in agreement with patient studies showing a connection between clinical outcomes and a robust coordinated adaptive response in which $CD4^+$ T cells, $CD8^+$ T cells, and antibodies are often required.[52]

Individuals with moderate COVID-19 showed evidence of productive innate and adaptive immunity, characterized by early transient increases in monocytes and NK cells, followed by sustained increases in memory T and B cells. Individuals with severe disease have exhibited symptoms suggestive of an immune response dysregulated by delayed and prolonged increases in Tfh cells, HLA-DRlo monocytes, and activated $CD8^+$ T cells.[46]

Adequate T cell homeostasis is required for successful viral clearance and clinical improvement.[50] Chronic viral infections have been cleared with the use of treatments aimed at reducing T cell exhaustion or death. Studies have shown that both IL-7, which increases T cell

self-renewal, and blocking of the inhibitory immunoreceptor-mediated interaction that suppresses T cell proliferation, such as PD-1/PD-L1, can promote antiviral immunity.[50] $CD4^+$ T cells specific to COVID-19 were rapidly induced in patients with acute COVID-19 and resulted in accelerated viral clearance.[53]

The role of leukomonocytes in COVID-19 viral clearance is not yet clearly defined, although previous studies have pointed out that suboptimal T cell and B cell responses can slow down viral clearance in patients infected with MERS-CoV and SARS-CoV. Lymphopenia was common in 25 COVID-19 patients, but after 2 weeks, the patients that cleared their infections presented restored numbers of $CD3^+$, $CD4^+$, $CD8^+$ T cells and B cells. The recovered patients had a higher count of leukomonocytes.[54]

In addition to T cell homeostasis, the cytolytic effects of NK cell function play an important role in COVID-19 clearance. NK cells that expressed receptor DNAM1 have been linked to more rapid recovery.[55] As NK cells play a key role in the innate immune system's viral clearance, a decrease in their populations may lead to a reduction in COVID-19 viral clearance.[54]

Future Research

As shown in numerous studies, the role of T cell performance in COVID-19 is crucial to viral clearance. Transfusions of $CD4^+$ and $CD8^+$ T cells may prove very rewarding in clearing COVID-19. Additionally, Th1 levels are important to regulate in a clinical setting because too high or too low levels of Th1 may contribute to COVID-19 pathogenesis. To decrease morbidity due to hyperinflammatory states from increased expression of TLR-1, TLR-7, and increased pro-inflammatory cytokines such as IL-6 and TNFα, these factors may need monitoring and even partial inhibition. Many factors of COVID-19 remain unclear and further studies into specific immune responses would be helpful in finding effective treatments for viral clearance.

Post-Acute Sequelae of COVID-19

Shortly after the beginning of the COVID-19 pandemic, a phenomenon known as "long-COVID," or Post-Acute Sequelae of COVID-19 (PASC), appeared to medical professionals.[56] On average, COVID-19 symptoms are resolved in 1-4 weeks, making patients with symptoms lasting longer than 28 days candidates for PASC diagnosis. Many studies have found that a large number of COVID patients report symptoms lasting longer than 28 days.

One study performed in early 2021 based out of the University of Washington surveyed 177 COVID-19 positive individuals between 3 and 9 months after symptom onset.[56] It was found that about 30% of outpatients reported persistent symptoms, corroborating an earlier study that found that 36% of outpatients had not returned to baseline health by 14 to 21 days after infection.[57]

These early studies were limited by small population sizes. A later international study analyzing self-reported COVID-19 symptoms from 4,182 patients found that 13.3% of participants reported symptoms lasting 28 days or longer; 4.5% reported symptoms lasting longer than 56 days; and 2.6% reported symptoms longer than 84 days.[58] The same study found that PASC was characterized primarily by symptoms including fatigue, headache, dyspnea, and anosmia; and that early disease features were generally predictive of the duration of symptoms.

Overall, these studies support the belief that PASC symptoms were linked specifically to past COVID-19 diagnoses. Although they disagree somewhat about the proportion of COVID-19 patients that develop PASC symptoms, it is clear that the experience of "long-COVID" is prevalent in at least some notable percentage of COVID-19 patients. Since then, many further retrospective studies have been published that highlight a high incidence of PASC symptoms in about one third of COVID-19 patients (see Table 1).

Table 2. Studies Reporting Long-COVID Data and the Percent of Outpatients with Persisting Symptoms.

Date	Study size	Mean population age (standard deviation)	Gender	Percent of outpatients with persisting symptoms	Source
May 2020	350	Median: 43	F: 53% M: 47%	36% (14-21 days)	[57]
March 2021	177	48	F: 57.1% M: 42.9%	32% (median: 169 days)	[56]
April 2021	4,182	45.97 (15.8)	F: 71.5% M: 28.5%	13.3% (28+ days)	[58]
July 2020	143	56.5 (14.6)	F: 37% M: 63%	87.4% (Mean 60.3 days [SD: 13.6])	[59]
Sep 2021	106,578	39.4 (18.4)	F: 58.4% M: 41.6%	36.55% (90-180 days)	[60]
Feb 2022	5,080,312	See source	F: 61.2% M: 38.8%	35% (31-150 days)	[61]

Potential Causes

The proximal cause of PASC is unknown and studies disagree about the most likely triggers. Some studies suggest that a cytokine storm of inflammatory cytokines causes tissue damage to major organs, causing PASC symptoms. On the other hand, one study done on patients with pneumonia secondary to COVID-19 infection suggests that persistent symptoms may be attributable to "biopsychosocial" effects of COVID-19.[62] Still, many studies dispute the possibility that PASC has a single proximal cause and instead attribute the range of PASC symptoms to a myriad of differing pathological traits of the virus.[63]

The Cytokine Storm and Hyperinflammation

The phenomenon of the cytokine storm, or hypercytokinemia, is not unique to COVID-19 infections, although it is a commonly cited cause of COVID-related mortality.[64,65] In essence, hypercytokinemia is characterized by three markers:[66]

1. Perpetuated activation of lymphocytes and macrophages causing immune dysregulation
2. Large secretions of cytokines caused by such perpetuated activation
3. Overwhelming systemic inflammation and multi-organ failure with high mortality

Early in the pandemic, high levels of inflammatory cytokines were observed in patients with poor outcomes. Especially noteworthy is the upregulation of IL-6, which has been correlated with poor COVID-19 prognosis in a large study of 1,473 patients.[67] In fact, serum concentrations of IL-6 above a threshold range from 35 to 80 pg/mL have been correlated with a substantially higher likelihood of mortality (Figure 2).[68,69] ICU patients were also found to have higher plasma concentrations of the proinflammatory cytokines IL-2, IL-7, IL-10, GSCF, IP-10, MCP1, MIP1A, and TNFα, compared to non-ICU patients.[30] This, coupled with a spike in other inflammatory markers such as enhanced concentrations of C-reactive protein, have led many researchers to conclude that COVID-19 mortality is strongly correlated with hyperinflammation.[70]

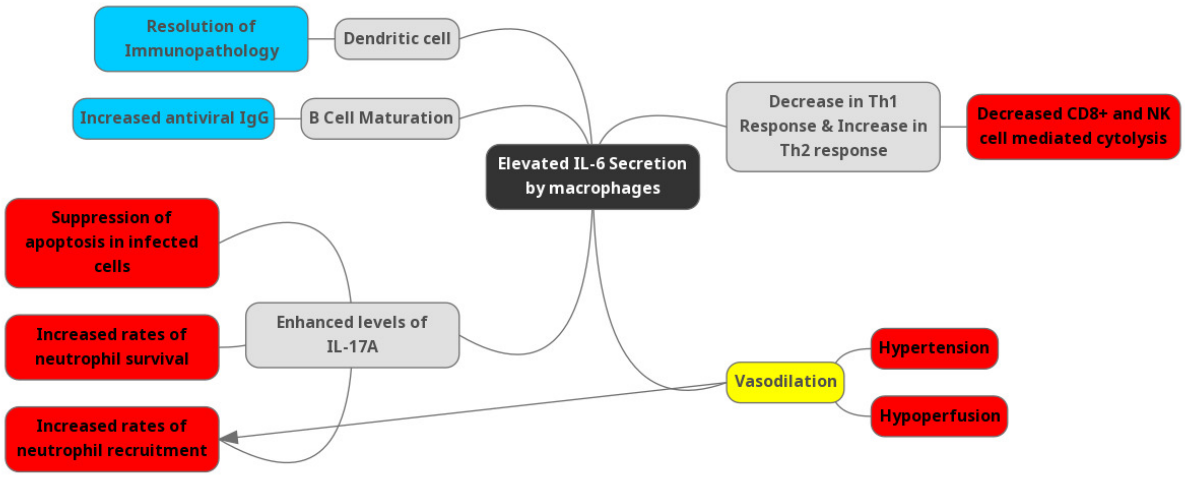

Figure 2. *Disproportionate upregulation of IL-6 is correlated with poor patient outcomes.*

Additionally, many PASC symptoms are consistent with inflammatory organ damage. For example, one study by the CDC surveyed more than 900 hospitals and found that the risk of myocarditis for COVID-19 patients was, on average, 15.7 times higher than for patients without COVID-19.[71] Another study conducted in Germany on 100 individuals recovering from COVID-19 (median 71 days after diagnosis) found that 71% of these patients had elevated levels

of troponin in their heart tissue.[72] The same study found that 78% of patients had abnormal results from cardiovascular magnetic resonance imaging.

Identifying hypercytokinemia as the proximate cause of PASC explains the myriad of symptoms associated with Long-COVID. Since cytokines are prevalent in circulation, they are able to access many different organ systems. As a result, a hypercytokinemia-related prognosis may result in a variety of symptoms depending on the patient's own physiology. Supportive of this is a relatively rare complication of COVID-19 infection; multisystem inflammatory syndrome (MIS). COVID-related MIS is characterized by a cytokine storm secondary to COVID-19 exposure.[73]

Myalgic Encephalomyelitis

A growing body of evidence suggests that the phenomenon of Long-COVID may be closely related to another condition; myalgic encephalomyelitis (ME). Also known as chronic fatigue syndrome (CFS), ME is a debilitating illness that has been well documented to affect millions worldwide. Especially noteworthy is the fact that viral infections are one of the leading known causes of ME.[74] Although the exact pathology of ME is poorly understood, it has been proposed that the mechanism involves many different body systems in response to the stress of severe infection.[75] Particularly well documented are the interlinked effects on the vascular system, intestines, endocrine axes, and thyroid hormone function (Figure 3).

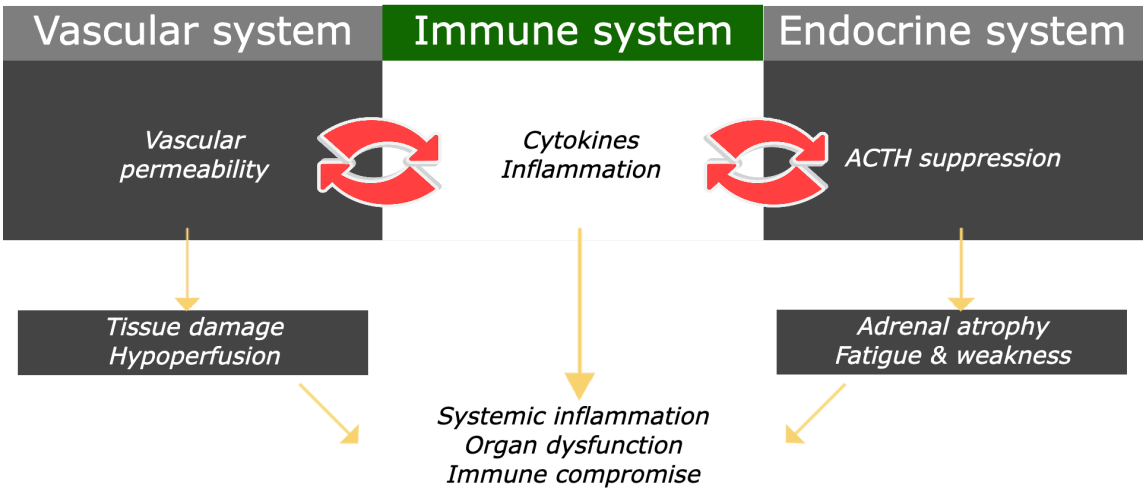

Figure 3. *Vascular and endocrine "vicious cycles" associated with myalgic encephalomyelitis.*

Research done on these interlinkages highlight the role of cytokines and inflammation in creating "vicious cycles" that may explain the chronic nature of ME. For example, vascular permeability is subject to an IL-6 mediated positive feedback loop characteristic of septic hypoperfusion.[76] Similarly, pituitary activity is suppressed by cytokine activity. Among other effects, cytokines suppress the pituitary release of adrenocorticotropic hormone (ACTH).[77] Since ACTH stimulates

adrenal function, prolonged cytokine activity on the pituitary results in excessive inflammatory responses.[78]

Fundamentally, myalgic encephalomyelitis is a multi-organ system condition perpetuated by positive feedback loops involving cytokine-mediated inflammatory reactions. This description draws close parallels with post-COVID MIS. The similarities of PASC to ME are striking, with a significant overlap in symptoms including lasting fatigue, unrefreshing sleep, and brain fog. Further strengthening this comparison is an open letter published in the British Medical Journal in August 2021 highlighting that approximately 25% of COVID patients developed ongoing symptoms that meet the diagnostic criteria for ME.[79]

Other Explanations

Another hypothesis is that hypercytokinemia cannot be the driving cause of PASC since a cytokine storm may be necessary for viral clearance. This is substantiated by the fact that IL-6 levels are lower in COVID patients than in other inflammatory conditions like acute respiratory distress syndrome or bacterial sepsis.[80] Additionally, hypercytokinemia—as indicated by elevated levels of IFN-γ, IL-6, IL-1, and TNF-α—is a documented symptom of other viral conditions, including H1N1 influenza.[81] In fact, the H1N1 cytokine storm was associated with inflammatory pulmonary compromise and mortality, similar to COVID-19 pathology.[81] The key difference is that recovered H1N1 patients reported persisting symptoms infrequently compared with that of recovered COVID-19 patients.[82]

Another hypothesis disputes the designation of PASC as a condition at all. One article attributed the phenomenon of Long-COVID to the "biopsychosocial" effects of COVID-19.[62] This study found that although 86% of 134 COVID-19 pneumonia patients discharged from the hospital reported residual symptoms on follow up, none of these patients had detectable radiographic abnormalities at that time. Essentially, these researchers concluded that Long-COVID was a psychosomatic condition.

These explanations are limited in their scope. The study concluding that PASC symptoms are due to biopsychosocial effects of the COVID-19 pandemic made that assertion despite the fact that only COVID-19 pneumonia patients were included in the study. As a result, the lack of radiographic abnormalities does not eliminate the possibility of PASC symptoms resulting from other organ system abnormalities. The position that hypercytokinemia is necessary for viral clearance relies upon the assumption that cytokine storms may not both be necessary for clearance and potentially detrimental to patient outcomes. More research is needed in this area to fully elucidate the etiology of PASC.

With this in mind, most evidence does suggest that hypercytokinemia plays a prominent role in an inflammatory reaction resulting in multi-organ damage that has been associated with the

Long-COVID phenomenon. ME and COVID-related MIS are strikingly similar, supporting the likelihood that PASC secondary to multisystem organ damage is triggered by mechanisms similar to ME. The correlation is close enough to suggest that PASC should be designated as a particularly virulent subcategory of ME symptoms that are specific to COVID-19 pathology.

Treatments of COVID-19

Treatments of Acute COVID-19

There are several different treatment options for COVID-19, and these fall into three major categories: drug repurposing, monoclonal antibody treatment, and new drug development.[4] This, of course, does not include indirect treatment methods with which COVID-19 is being treated, including oxygen therapy, steroids, immunosuppressors, or vaccines which reduce the spread of infection.[83]

Drug repurposing, also termed repositioning, is the use of drugs approved to treat one disease for the purpose of treating another.[84] Since drug repurposing utilizes substances which have been thoroughly studied, with well-known pre-clinical, pharmacokinetic, and pharmacodynamic profiles, the drug can be fast-tracked through to phase 3 human clinical trials.[85] This makes the drug discovery and approval process faster, cheaper, and largely more reliable. Remdesivir is one such drug which has been repurposed towards the treatment of COVID-19.[86] Remdesivir is an antiviral drug originally developed as a treatment for Ebola virus disease and which functions by interfering with viral RNA-dependent RNA polymerase activity.[87] Clinical trials have shown remdesivir to be effective for the treatment of COVID-19, and, although the full efficacy of this treatment is still being investigated, the FDA granted emergency use authorization of remdesivir for patients with severe COVID-19 and most recently expanded use of remdesivir treatment to outpatients with mild-to-moderate COVID-19 disease.[88] Further investigation into the use of remdesivir, as well as of other repurposed drugs such as ivermectin, lopinavir/ritonavir, and chloroquine (hydroxychloroquine) for the treatment for COVID-19 is ongoing.[4,88,89]

Another promising type of treatment for COVID-19 involves the use of monoclonal antibodies. Several monoclonal antibodies have already been developed, including those which target the spike protein on the virus and the receptor binding domain (RBD), which is used by the virus to bind to host ACE2 and enter the host cell.[1,91] These include sotrovimab, bamlanivimab, etesevimab, asiriviamb, and imdevimab, among many others.[91] Monoclonal antibodies may also be used to control the cytokine storm, and these include clazakizumab, siltuximab, levilimab, and adalimumab, among many others.[91] The full efficacy of monoclonal antibody treatment is yet to be fully elucidated, as there are several concerns with this treatment modality. These include production constraints and susceptibility of the treatment to virus mutation.[4] However, with ongoing research and the continual fine tuning of treatment criteria or indications, there is much promise that this will continue being a successful treatment option.

The development of an oral drug that patients may take at home is another avenue showing great promise. The ability to take a drug at home would allow patients to receive treatment at the early stages of infection, and thus reduce the number of hospitalizations and subsequent mortality.[4] In fact, these are the exact results seen in a phase 3 clinical trial of molnupiravir (EIDD-2801), an oral drug produced by Merck which functions similarly to remdesivir by disturbing the activity of viral RNA polymerase.[92,93] Pfizer produces the second class of oral drug which is becoming available in the treatment of COVID-19, called paxlovid (nirmatrelvir-ritonavir).[94] Paxlovid functions differently than molnupiravir, behaving as a protease inhibitor which disrupts virus replication.[95] These two oral medications will continue being studied intensively as their use becomes more widespread.

Treatment of Post-Acute Sequelae of COVID-19

The likely implication of hypercytokinemia in Long-COVID has led physicians to attempt to treat these conditions with immunosuppressive agents. In particular, Tocilizumab has been used in treatment because of its effects as an IL-6 antagonist.[68] Tocilizumab treatments have proven to improve clinical outcomes for patients with severe COVID-19.[67,96]

The success of tocilizumab therapy must be tempered by acknowledging the dangers associated with immunosuppressive treatments. In weakening the immune response to COVID-19, immunosuppressive treatments may open the door to additional infection. As a result, this treatment is currently only approved for patients with severe cases of COVID-19 in which recovery is unlikely without extreme interventions.

Convalescent plasma therapy (CPT) is another treatment that has proven successful in the management of severe COVID-19 cases.[97] In combination with tocilizumab therapy, CPT was found to reduce plasma IL-6 levels much faster than either therapy on its own.

One of the downsides to the similarity of ME and PASC pathology is that there are likely no treatment possibilities other than symptom management. Instead, emphasis should be placed on treating seriously ill COVID patients with preventative immunosuppressive therapies like tocilizumab in the most severe cases, or less invasive CPT treatments when available. Unfortunately, many non-ICU patients that would not be eligible for tocilizumab treatment report PASC symptoms. Similarly, CPT should be reserved for severe cases because overuse of CPT may have the effect of forcing the mutation of new COVID-19 strains.

Conclusions

In conclusion, our review demonstrates that COVID-19 is a multi-phasic disease, with multiple phases of infection drawing responses from the host immune system corresponding to the stage of infection. This detail has been paramount in the tailoring of treatment options for patients with COVID-19 and will continue being paramount as more treatments are developed and brought

into clinical practice. Our review also describes areas where future research is needed and will become fundamental to understanding the body's immune response to COVID-19.

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*****This paper has been previously published in the International Journal of Molecular Sciences August 2022.

Yeshiva University Journal of Medicine and Dentistry

Review

The Advantages, Manufacture, and Applications of Bispecific Antibodies

Rachel Kaplan

Abstract

Bispecific antibodies (BsAbs) are antibodies that have two binding sites with different specificities. BsAbs provide advantages over monospecific antibodies, such as increased binding site specificity, better recognition of single antigens with multiple epitopes, and the ability to block two different antigens simultaneously. They address the issue of patients developing resistance to antibody treatment and can reduce expenses associated with multiple monospecific antigens. BsAbs can also recruit one cell type to another, creating a powerful tool for immunotherapy. Bispecific antibodies were first created in the 1960s by Nisonoff and his collaborators, and over time, a multitude of methods were developed to engineer BsAbs. The manufacture of BsAbs is particularly difficult due to the variabilities in recombination of their four polypeptide chains. The knob-in-hole technique is one of the most well-known methods for BsAb engineering, but it has limitations. The SEED method, along with other techniques, address some of these limitations. Three types of BsAbs are commercially available today to treat cancers, yet research in this field continues to advance.

Introduction

Antibodies are crucial components of the immune system. These specialized, Y-shaped proteins are excellent "scouts" for potential pathogens. They search for and either destroy antigens directly or tag the antigens through the use of unique binding sites. While naturally occurring antibodies have an extensive range of antigen-binding center variants, each individual antibody is monospecific. In other words, the two binding sites located on the tips of antibodies are identical.[1] Bispecific antibodies, or BsAbs, are artificially manufactured antibodies that contain two binding sites with different specificities, whether that be to different antigens or to different epitopes on the same antigen.

Advantages of Bispecific Antibodies

Bispecific antibodies provide a number of advantages over monospecific antibodies, as they provide more binding site specificity due to their ability to recognize and bind to two different surface antigens. It has been found that many tumors coexpress more than one surface antigen, requiring more than one monospecific antibody. Oftentimes, to approach these types of pathogens more efficiently, one bispecific antibody can be used.[2] Further, if a single antigen has more than one epitope, bispecific antibodies can allow for more effective recognition of that

antigen. It is known that single-target antibodies do not sufficiently destroy tumor cells due to the complex signaling pathways and mechanisms associated with many cancers. BsAbs can pose a solution to this issue because of their ability to block two different antigens involved in pathogenesis simultaneously.[3] Bispecific antibodies are especially compelling because of their ability to recruit one cell type to another, such as their ability to target proteins on cancerous B-cells while the other recognition site can recruit immune cells, such as killer T-cells in order to detect and destroy the cancer (Figure 1). BsAbs also address the issue of patients developing resistance to antibody treatment. Due to their increased specificity, BsAbs can help to avoid the development of immunity to treatment.[4] The use of BsAbs can potentially reduce expenses associated with and enhance the development of clinical trials that might otherwise use multiple monospecific antigens.[2] BsAbs can also be useful in creating bridges to combine two protein complexes, which expands their relevance beyond immunotherapy.[5]

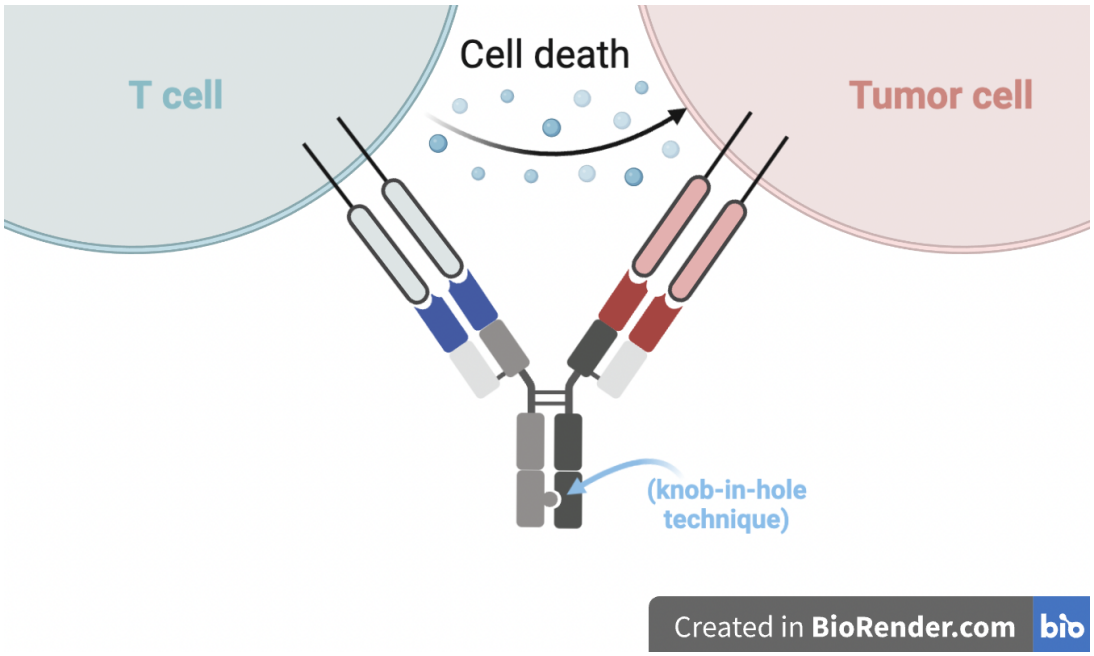

Figure 1. *Bispecific antibody recruiting T-cells and targeting tumor cells.*

Early Development of Antibody Engineering

Bispecific antibodies were first created in the 1960s by Nisonoff and his collaborators. They combined antigen-binding fragments from two different immune cell mixtures in solution and found that they reassociated into "hybrid" antigen-binding fragment complexes.[6]

In 1975, Milstein and Köhler developed a technique for the production of large amounts of monoclonal antibodies through the use of hybridomas, which was later applied to the synthesis of bispecific antibodies (Figure 2). By fusing myelomas, or cancerous forms of plasma cells that do not produce antibodies, with wild-type plasma cells that can produce antibodies, hybridomas are formed. These 'fusion cells' combine the longevity of myelomas with the

antibody-producing properties of plasma cells to allow for extensive production of antibodies.[7] Through the fusion of two separate hybridomas to form a quadroma, identical, bispecific antibodies can be formed in extensive amounts.[8] It was also discovered that these hybrid antibodies can be artificially synthesized by directly chemically conjugating two monospecific antibodies. Over time, a multitude of methods were developed to engineer bispecific antibodies with unique biochemical properties, which allowed for a platform to create potential immunotherapies that be curated to fit specific pharmacological needs.[6]

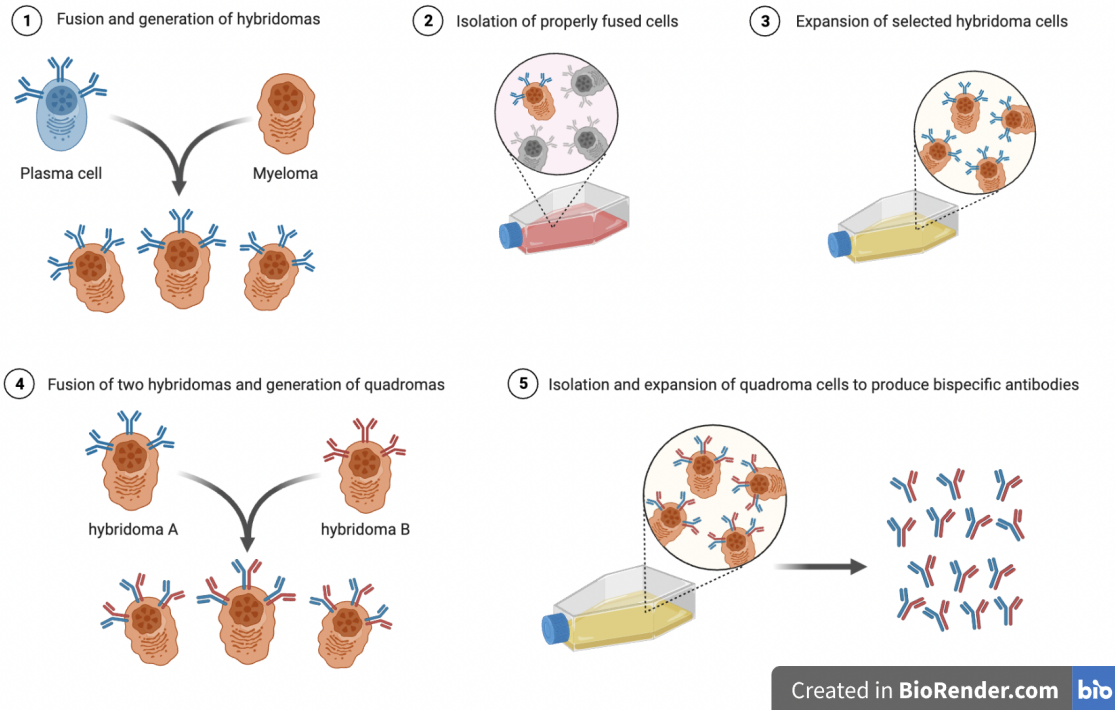

Figure 2. *Production process of creating bispecific antibodies through the use of hybridomas.*

Limitations of Bispecific Antibody Development

The development of bispecific antibodies poses a certain challenge due to their molecular makeup and the variabilities in the recombination of their four polypeptide chains. Standard, naturally occurring antibodies are made up of two identical heavy polypeptide chains and two identical light polypeptide chains. These chains are held together with disulfide bonds. At the ends of the Y-shaped structure, there are two identical antigen binding fragments, referred to as the Fab region.[9] Naturally occurring antibodies are monospecific, bivalent, and symmetrical, due to their makeup of two identical heavy chains and two identical light chains. In order to create bispecific antibodies, one heavy and one light polypeptide chain from one type of antibody must be chemically combined with a heavy and light chain from another antibody. Unfortunately, this recombination process results in the formation of undesirable protein complexes. Theoretically, the recombination of heavy and light chains from two different antibodies can result in 16 different complexes, only one of which is bispecific with the others either monospecific or nonfunctional.[10] Even the hybridoma technique results in numerous, non-functional side

products, as the hybridoma cells produce two different heavy and two different light chains, allowing for the polypeptide chains to associate in a variety of ways.[6] Purification of the bispecific antibodies from these recombinants is not readily available as is difficult and expensive.[11] To combat this issue, many biochemical techniques have been engineered to allow for preferential formation of functional, bispecific antibodies.

Methods of Bispecific Antibody Development

One of the most well-known methods for bispecific antibody engineering is the knob-in-hole technique. This technique was originally developed by Crick in 1952 in order to pack amino acid side chains within alpha helix structures. In 1996, John B. Ridgway and his colleagues applied the knob-in-hole method for the adjustment of protein structure to the manufacture of heterodimer antibody proteins. Essentially, this technique replaces a small amino acid in the C_H3 region of the heavy chain with a larger amino acid, creating a protrusion in the protein dimer, or a "knob". In the other heavy chain (with different antibody specificity), a large amino acid in the C_H3 region is replaced with a small amino acid, creating a "hole". This method structurally facilitates the polypeptide chains with proper recombination. This technique allows for a recombination efficiency of 57%. The knob-in-hole technique is an incredibly novel method to enhance the formation of BsAbs (Figure 1). Still, it has its limitations. Due to the change in amino acids, the stability of the protein structure is negatively affected. Therefore, other methods to create these bispecific antibodies were investigated.[12]

In 2009, Jonathan H. Davis and his colleagues created fusion antibody proteins using a method called strand-exchange engineered domain (SEED). This team aimed to improve upon the classic knob-in-hole approach, as it was found that to optimize the stability of BsAbs made with this technique, a non-native disulfide bridge between the heterodimers was required.[13] To avoid the introduction of an external disulfide bond, the SEED technique to create a more stable heterodimer product was investigated. Two protein derivatives were manufactured from Immunoglobin G and Immunoglobin A C_H3 domains. The two derivatives of these parent polypeptides were called AG and GA SEED C_H3 domains. This pair of SEED C_H3 domains preferentially combine to produce heterodimer antibody proteins. Davis and his team created two different bispecific antibodies using the SEED method as models.[11] This method is particularly significant as it maintains the binding affinity of bispecific antibodies in comparison to their parent, homodimer proteins and allows for the manufacturing of biochemically stable products.[14]

Applications in Medicine and Immunotherapy

Today, three types of bispecific antibodies are commercially available for treatment[15] and over 90 BsAbs are currently being investigated in pre-clinical and clinical trials.[2] Catumaxomab, one of these commercially available BsAbs, treats tumor cells by recognizing the EpCAM antigen. It also has specificity for the CD_3 antigen on T cells, which, upon binding, stimulates T cells to secrete cytokines and promotes the elimination of tumor cells. Essentially, this BsAb recruits

pathogen-fighting T cells directly to the site of the cancerous cell. Blinatumomab, another commercially available BsAb, helps to treat precursor B ALL leukemia due to the Philadelphia chromosome, which is notorious for its complications and difficulties in treatment. Patients with this type of leukemia always have some measurable residual disease even after extensive treatment, which often causes relapse. The Blinatumomab hybrid, by recruiting T cells to the antigen, has been shown to cause some degree of complete remission in patients. This is especially significant as leukemia is referred to as a liquid tumor, which means that the cancerous growth can not be surgically removed or easily identified. The Blinatumomab is helpful in combating this disease as it is able to locate and tag cancerous cells to promote antibody-directed cytotoxicity. The third type of BsAb commercially available is called Emicizumab, which is used to treat patients with congenital factor VIII deficiency, or hemophilia. People without this disease are able to produce three clotting factors: factor FIXa, factor FX and factor VIII. Factor VIII, which hemophilic patients lack the ability to produce, will gather factors FIXa and FX to form a coagulation complex protein and stop blood flow. Emicizumab has specificity for both factors FIXa and FX and links these two proteins together, which is effective at creating a protein complex that causes blood clotting. Emicizumab is especially interesting as it acts as a protein linker to provide treatment instead of interacting with immune system cells.[15]

Conclusion

Bispecific antibodies have emerged as promising therapeutic tools for the treatment of various diseases, including cancer, autoimmune disorders, and infectious diseases. Their ability to be manufactured to meet the needs of a particular disease or match the specificity of a multitude of antigens and other proteins allows them to be incredibly versatile. They offer several advantages over traditional monospecific antibodies, such as the ability to simultaneously target two different antigens, their enhanced resistance to immunity, and the ability to treat complex diseases with multiple pathogenic pathways. As research in this field continues to advance, it is likely that bispecific antibodies will play an increasingly important role in the development of new and effective treatments for a wide range of diseases.

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Yeshiva University Journal of Medicine and Dentistry

Review

The Risks and Benefits of Titanium Medical Implants Through the Conscious Prevention of Osteoporosis

Noa De Louya

Abstract

Titanium medical implants have changed the lives of many individuals suffering from osteoarthritis, missing teeth, spinal injury, scoliosis, hip injuries, knee injuries, and many other bone fractures or bone related diseases. However, studies have shown that osteoporosis in patients discourages many physicians from proceeding with surgeries involving titanium medical implants due to the high risk of bone fracture post surgery and low osteoperotic bone resorption.

This complication is especially relevant as numerous factors, including longevity due to exponential medical advancements, unhealthy fitness cultures and generational ideals, can increase vulnerability to osteoporosis. In turn, if society is conscious about these factors and actively implements ways to prevent osteoporosis, patients can take advantage of these metal medical implants. Nevertheless, some concerns have been raised about titanium medical implants and the effects they may have on DNA via corrosion. This certainly does not mean discontinuing the use of these implants, but educating patients on the potential risks allows for an informed discussion with the physician on whether the implant is needed or if there are suitable alternatives. Additionally, teaching the patient the necessary precautions to improve their bone health facilitates the physician's decision and allows him to be more efficient with his time. Furthermore, ongoing research on these popular implants, brings the medical community one step closer to finding more effective strategies for preventing the potential risks of titanium metal as well as identifying more suitable materials. Following the discussion on osteoporosis relevance, this review will examine the toxicological effects of titanium medical implants and assess whether literature considers them to be negligible in the larger context. Robust research studies have concluded that the benefits of metal medical implants outweigh the risks and should inspire people to be precautious of their bone health in order to take advantage of what these implants have to offer.

Introduction

Osteoporosis is a degenerative bone disease that occurs when bone density decreases, leading to brittle and weak bone that is more susceptible to fractures.[1] While a myriad of factors can contribute to the development of this disease[2], this review focuses on the impact of age, diet and physical activity, gender (in terms of hormonal changes), and drug misuse.

Age

With the exponential medical advancements, including vaccines, antibiotics and medical technology, longevity has significantly increased. Unfortunately, a longer life does not slow down the natural degeneration of bone, thus one may be more susceptible to osteoporosis.[3]

Gender/hormonal changes and lifestyle (physical activity, diet, drug misuse, alcohol)

Women are naturally more vulnerable to osteoporosis due to menopause, during which estrogen levels are significantly lower leading to degeneration of the bone.[1] On the other hand, although it is possible for men to suffer from osteoporosis with time, it is generally more prominent when it is self-induced. In recent years, men may have become more susceptible to osteoporosis due to the huge surge in "fitness culture", "weightlifting culture" and consciousness of body image. Although there has been a healthy increase in awareness of physical health after COVID-19, social media platforms have allowed many inexperienced individuals to promote over-exercising[2] and unattainable physical goals that require unhealthy habits, like steroid uptake,[1] which has also been shown to increase susceptibility to osteoporosis. Research confirms that too little exercise or over-exercising[4] in high contact sports or workouts can be highly damaging to bones and joints.[2] However, correct and well practiced exercise can, on the contrary, be beneficial and reparative for individuals with osteoporosis.[4] Additionally, smoking and drinking (factors contributing to osteoporosis)[3] have become more normalized through social media platforms which offer a more efficient and attractive way to promote these activities, to all age ranges.

Osteoporosis and metal-bone replacement surgery

When a metal implant is placed within a bone, it must first be stabilized by the bone itself and proceed with osseointegration[5], which is when the bone tissue fuses onto the metal implant anchoring it into the original bone, another term for this phenomenon is "biological fixation."[6] However, when the bone is osteoporotic, there is a 25% bone mass decrease and its regeneration[7] and osseointegration abilities plummet.[8] This results in a major increase in marrow space which makes the bone fragile and it is no longer strong enough to support a metal implant.[7] Unfortunately, fracture reconstructive habilitation has become a great challenge for surgeons.[9]

In the case of hip implants, the metal shell fits into the pelvis and a ceramic ball that is attached to a metal piece is inserted into the thigh bone.[10] A study done by Bottai, Vanna et al. tested the three potential complications: "perioperative fracture, an increased risk of periprosthetic fracture, and late aseptic loosening" in patients needing a total hip arthroplasty after unsuccessful medical therapy.[5] One of these patients had osteoarthritis which is a disease that destroys the joints. As osteoarthritis is present in older patients, the aging process led to osteoporosis as well. In fact, 74% of female OA patients have osteoporosis simultaneously.[5] Consequently, the surgeon is unable to proceed with the surgery and has to wait until the patient regains bone health. This time interval offers more opportunity for further complications. Figure 1 depicts an X-ray of a periprosthetic fracture in an osteoporosis patient.

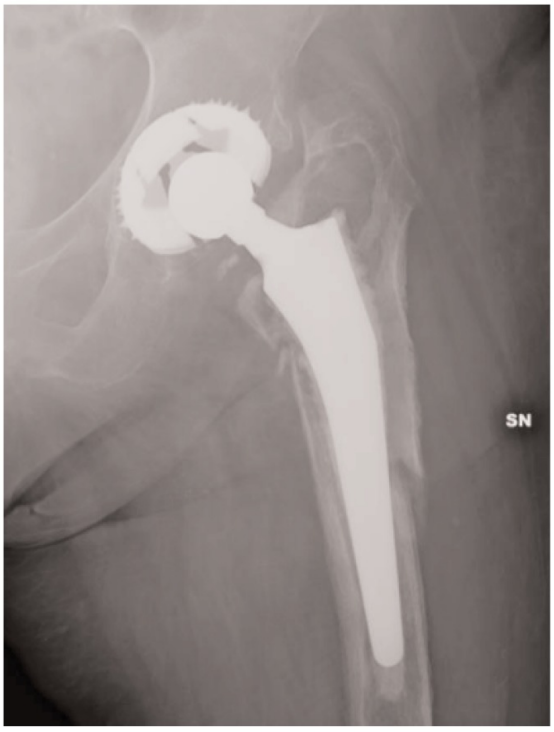

Figure 1. *Periprosthetic fracture in an osteoporosis patient.*[5]

The fracture can be noticed on the lower right of the femur bone. This was caused by the lack of support of the metal implant by the fragile femur bone.[5] Additionally, Figure 2 below depicts the aseptic loosening of the implant due to excess marrow space.

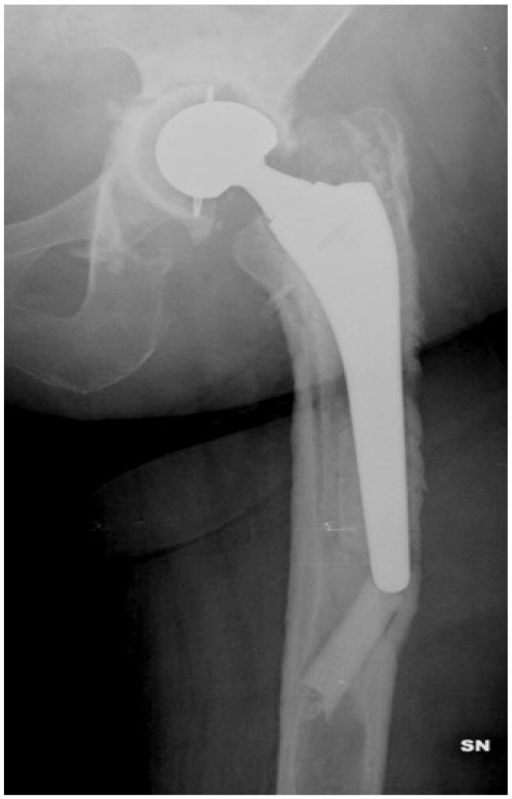

Figure 2. *Aseptic loosening in patient with hyperparathyroidism.*[5]

The study concludes by listing some of the factors that can lead to osteoporosis like chronic drug use, alcohol abuse, low calcium intake, ect. The results strongly indicate that physicians should conduct a thorough analysis of the patient's bone health before surgery,[11] such as doing densitometric scanning and bone density testing.[8] There should also be regular radiological surveillance of osteoporosis and conscious effort to improve it. Alternatively, if the physician judges that although the bone is osteoperotic, surgery is possible, then a longer implant is used.[12]

As discussed, osteoporosis can be a cause of implant failure, yet are there genotoxic and cytotoxic effects on the molecular level? Does titanium metal also affect the bone and its surrounding tissues?

Metal Medical Implant and Application

Medical implants are devices or tissues that are inserted inside of the body used to improve one's health and/or replace a function lost due to body malfunctions or accidents.[13] These medical devices should be corrosion resistant, biocompatible, bio-adhesive, bio-functional, processable and available to be innocuous in bodily fluids.[13] Some common materials are metals, ceramics, polymers, and composites. These materials are frequently used for cosmetic and health purposes.

Another common metal medical implant is the titanium hip implant. These implants are used as a replacement for damaged or worn out hip joints. Just like a human hip joint, the titanium implant consists of a titanium stem which is inserted into the femur with a metal ball at the top that fits into a titanium socket that is implanted into the pelvis, right where the ball-and-socket of a healthy individual joint would typically meet.[9] The implant thus has direct contact with the bone and the surrounding tissues, like muscle and connective tissue.[14]

Metal as a Medical Implant Material

Titanium metal is a popular material used in medical implants since it abides to the appropriate characteristics mentioned earlier which is supposedly harmless in bodily fluids. This metal is especially corrosion resistant and has excellent biocompatibility. However, titanium is a rare and costly material in its pure form, thus titanium alloys, like Ti-6AI-4V are mostly used in the metal medical implant industry. These alloys are composed of 90% titanium and 6% aluminum, 4% vanadium and traces of oxygen.[15] Regarding titanium joint replacements like hip implants, research also suggests similar potential risks as discussed. A study done by Gajski et al., suggest that the wear of hip implants composed of Ti6Al4V and CoCrMo can lead to debris that can accumulate in bone marrow and cause chromosome aberrations, given that the rod is embedded in the femur and the rest of the implants is adjacent to soft tissues.[16] The excess marrow space in osteoporosis patients may allow for a greater accumulation. The study focussed on analyzing materials commonly used in total hip joint replacements, in vitro models, via investigation of cytotoxic and genotoxic effects on human peripheral blood lymphocytes. After performing several experiments, like chemical characterization, induction of DNA strand breaks, micronuclei assay, and more, the results indicated that there was no significant genotoxic effect on human peripheral blood lymphocytes.[16] However, another test called particle morphology assessment that studies the physical properties and shape of particles, showed that the wear debris were very small, making them highly reactive to their surroundings since submicron particles have larger surface area. The wear debris can therefore easily interact with surrounding media and cause inflammatory responses and tissue damage. As a result of the wear debris, an interface between the bone and the implant is created. In turn, foreign- body granulation tissue response invades the available space causing inflammation which activates osteoclasts that ultimately break down the bone resulting in a progressive local osteolysis.[17] This effect eliminates the anchoring of the implant in the previously healthy femoral bone. In osteoporosis patients, induced osteolysis occurs in addition to the initial significant interface between the bone and the metal, leading to a possible grave fracture.[17] The study also mentions how this can increase metal ion levels in organ sites. Although the overall research suggests that there is no carcinogenic effect, some genotoxic tests are contradictory and the particle effect can also lead to negative effects, thus further parameters need to be addressed.[16]

Another review done by Coen et al., focuses on studying the effects of the debris of a specific concentration while the previously discussed article tested the actual concentration of debris in blood lymphocytes which was found to have no/minimal genotoxic effect.[18] In this study the initial stock solution had a dense titanium debris composition. The experiment consisted on culturing fibroblast cells in medium and exposing them to different concentrations of titanium debris which was extracted from a patient's periprosthetic tissue who underwent revision total hip arthroplasty. The higher dilutions had a lower indication of unstable aberration compared to higher concentration. Results of this experiment suggested that titanium debris can cause chromosomal aberrations. They found that this chromosomal instability was transmitted to the progeny of human fibroblast cells. Although this article suggests that there were significant cytotoxic/genotoxic effects, it may be based on the fact that the cells were directly exposed to higher concentrations of titanium debris.[18] As it was well researched, titanium metal has low corrosion rates and it would therefore be unlikely that the surrounding tissue be exposed to as high of a concentration. The verdict remains that, as the first article mentioned, the effects are negligible, however studies should be continuous on this topic.

The further section discusses these biological processes at a more biochemical level.

Metal Corrosion in the Body and its Genotoxic Effects

Ti-6AI-4V, Titanium alloy is generally known to have low corrosion rates, good biocompatibility, and low toxicity.[19] However, some studies have shown that the components of the alloy, like vanadium, can be more corrosive and can lead to illnesses like peripheral neuropathy, osteomalacia and Alzheimer's disease.[19] On the other hand, further studies suggest that titanium itself can corrode over time and ultimately cause severe inflammation to its surrounding tissues.[20]

Essentially, corrosion due to possible oxidation causes the release of metal ions, which are cations, shown to be non-biocompatible with the human body, particularly, toxic to the genome. These metal ions can cause small deviations from normal levels of oxidative stress that occur naturally by oxidative phosphorylation for which homeostasis cannot be restored. This unhealthy amount of oxidation can lead to acute and chronic toxicity, like cancer.[19] The corrosion of titanium oxide releases Ti^{4+} and $2O^{-2}$ into body fluids and tissues which offer easy access to DNA. Each of these ions react in particular ways that ultimately lead to DNA damage. The interactions of these ions and DNA will be discussed individually below.

Due to selective permeability of the membranes, the Ti^{4+}cations enter the cell through ion channels and are released in the body fluids and tissues surrounding the metal implant and the positively charged species reacts with the negatively charged backbone of DNA and its electron donor base pairs. The metal cation can interact completely with DNA bases leading to DNA breakage. Alternatively, the metal ion can partially bond to water molecules found in body fluids,

creating a complex ion that can hydrogen bond to DNA.[19] Water bonded to a metal ion, a complex ion, significantly influences strong hydrogen bonding leading to the rupture of hydrogen bonding between base pairs and ultimately, significant DNA breakage.[19]

As for the interaction of O_2 with DNA, oxidative damage can take place and disrupt the double helix structure.[21] The production of ROS, reactive oxidation species, can result from homolytic breakage of the oxygen atoms after the dissociation from the metal ion occurring through corrosion in biological systems.[21] Additionally, this oxygen ion can gain a single unpaired electron and become a free radical. Because the ROS are unselective and extremely unstable, they will react with the electron donor base pairs resulting in the formation of 8-hydroxyguinine.[19] Subsequently to a radical substitution reaction, another homolytic fission can take place creating a radical OH group bonded to base pairs which can react un-selectively with all the components of the DNA molecule. Purines and pyrimidines will be damaged and lose their original structures. This rearrangement eventually alternates DNA physiologically.[19] The mutations resulting from this malfunction cannot be repaired by checkpoint proteins, for instance DNA polymerase, given that the proteins have also been inhibited by metal ions.[19]

The further section discusses in more detail the direct damage these processes cause and how DNA is damaged.

Analysis of DNA Damage in Association with Metal Corrosion

Essentially, DNA stores all genetic information. Once it is damaged or altered, error in DNA structure occurs, ultimately causing mutations that can have a detrimental effect on the cell's function.[19] In the case that this damage causes multiple abnormalities in the sequence of DNA, the cell proliferates uncontrollably and ultimately causes cancer.[19] However, cells have several mechanisms that intend to repair the damage, but in case that the damage is too severe to repair, these mechanisms trigger cell death, also known as apoptosis.[19] If these mechanisms are inhibited or do not function properly, the risk of disease increases since there is an accumulation of failed to repair errors in DNA structure or sequence. With all of this said, minimizing risks and attenuating factors that are responsible for DNA damage is vital to prevent diseases and malfunction of cellular repair mechanisms.[19]

Micronucleus assays and comet assays were performed to prove these genotoxic effects. The micronucleus assay evaluates DNA breakage and genetic alterations.[19] These malfunctions are monitored through the frequency of micronucleated cells. In an experiment, the frequency of micronucleation was determined in 1000 binucleated cells with a well-preserved cytoplasm. After observation, there was a difference in binuclei only at particularly higher concentrations, 75% and 100%, at the third treatment 25 and 26, respectively, compared to the control indicated to be 15.[19] The comet assay, another method of DNA damage determination, evaluates DNA breakage using electrophoresis. The number of DNA breaks are reflected through the

comparison of the comet's head and tail intensity. The comet assay indicated that vanadium creates DNA breakage in the liver, kidney, lungs, and spleen.[22]

Other methods of study were used to detect DNA damage indirectly given the relationship between metal ions and DNA damage.[23] A study done by Sedarat *et al.* (2001) used absorption spectroscopy to monitor the release of metallic ions from the Ti-6AI-4V alloy over a period of 96 days in a solution that simulated the composition of body fluids.[23] The study by Sedarat *et al.* (2001) showed that aluminum and titanium had a constant release of ions during the 96 days. However, vanadium released ions only on the first 6 days.[23] Additionally, a study performed by Vendittoli *et al* (2010), looked at the blood concentrations that showed various ions from these metallic devices.[14] The ion concentration in individuals with metal implants was significantly greater than individuals without the devices.[14] Although corrosion and ion release may occur, compiled results judge that the benefits of titanium implants surpass the overall effects.[5]

To avoid the problems discussed above, it is important to be aware of any unusual pain in the hip, groin or thigh, in the case of a hip replacement and report to a doctor for revision. Limited mobility, fever, inflammation may also be symptoms related to a failed replacement.[24]

Conclusion

Although life can get busy, it is imperative to make physical health a priority and incorporate activities that promote it in daily life. From patients with osteoporosis to those that already house a titanium medical implant, bone health monitoring should always be done, especially that implant survival rates have become as high as 15 years or longer. Complications with implants would require invasive revisions that can give opportunity for infections to arise. Consuming foods rich in calcium, vitamin D, protein and engaging in professionally taught physical activity consisting of weight-bearing exercise, all contribute to a better bone health.[25] Improving the lifestyle that this generation has instilled in many, that consists of bad habits like, smoking, alcohol consumption, insufficient hours of sleep and drug misuse, can lead to better outcomes with metal implants and most importantly make the research on these implants worthwhile. While advancements in medical research that contribute to longevity are impressive, it is our responsibility to deal with the downsides that may arise from these innovations.

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Yeshiva University Journal of Medicine and Dentistry

Review

Genotoxic Effects of Chemotherapy

Aviva Itskowitz

Abstract

Genotoxic substances are present everywhere and are unavoidable. This poses a large risk due to their ability to cause great damage to a person's DNA. Unfortunately, this may lead to an increased risk of the development of cancer cells within an organism. If the cancer caused by these genotoxic chemicals is left untreated, it will largely decrease a person's quality of life and will often lead to fatality. Although chemotherapy is a very strong and often successful form of cancer treatment, the drug poses many risks within the realm of genotoxicity. This study aimed to evaluate the risks posed to both patients and the healthcare providers in order to determine the overall value of this treatment. A major genotoxic risk that arises from the use of chemotherapeutics is the possibility of secondary cancers arising. For example, Myelodysplastic Syndromes and Acute Myeloid Leukemia are very common secondary cancers. It was found that there was a significant increase in DNA damage and numbers of abnormal cells after chemotherapy treatment. In addition, genetic lesions occurred, adding to the overall genetic instability and risk for future therapy-related malignancies. The study also explored the different routes in which chemotherapy proves toxic to a person's DNA: directly and indirectly. After gaining a full understanding, multiple prevention ideas were explored. Treatment with DNA neutralizing agents and the relationship between nutrition and chemotherapy-induced toxicity were considered in this study. It was found that due to the fact that normal cells and cancerous cells react differently to fasting, intermittent fasting may have positive effects for cancer patients being treated with chemotherapy. Epigenetic reprogramming was explored to fully grasp the influence diet can have in chemotherapy treatment. The use of ketogenic diets (KD's) in cancer treatment was also explored. The use of tyrosine kinase inhibitors (TKIs) combined with fasting was also explored to understand if it could enhance the efficacy of cancer treatment. Although many of these studies produced promising results, further research must be done to establish these results.

Introduction

Over a lifetime, humans interact with many external chemicals present in the environment. Many of these chemicals cause significant damage to the DNA of these organisms by causing DNA mutations which lead to an increased risk of cancer.[1] These mutations can be assessed using assays, or tests that measure the biochemical activity of a substance. The results of these assays indicate the overall impact that a genotoxic chemical has on a specimen. Given the level of exposure that humans have to genotoxic chemicals, it behooves scientists to acknowledge the

devastating effects of these substances, attempts to regulate human exposure to them, and work to better understand their mechanisms of action to limit their impact.[1]

While damage to DNA is unwanted, the main treatment for cancer utilizes these effects of toxic substances in a positive manner. Chemotherapy is a drug treatment that is "administered to inhibit the growth of cancer cells, kill cancer cells, or block cancer cell proliferation".[2] These drugs achieve this goal by interfering with the RNA or DNA of the patients' growing cancer cells. Chemotherapy treatments are costly, timely, often rigorous, and have many side effects. Despite these drawbacks, the survival rate of cancer patients around the world has greatly increased due to improvements in this method of treatment. Chemotherapy has either added years to cancer patients' lives or eradicated their cancer completely, enabling the patient to thrive and live a "normal life".[3,4,5]

At first glance, these chemicals would be great assets in treating terminal illnesses. However, while these drugs do interact favorably with cancer cells, they do have negative effects on healthy cells in patients' bodies. Additionally, the long-term exposure of oncologists who deal with these substances daily can cause DNA damage to them as well, even though the drug is not being administered to them directly. Supported by research that has shown that chemotherapeutics have induced genotoxic effects on those healthcare providers administering the treatment to patients, it is essential for those administering to learn proper techniques to minimize danger to themselves and the patient.[6]

Although there are over one-hundred types of chemotherapeutics, they can be divided into seven main categories of drugs: alkylating agents, nitrosoureas, anti-metabolites, plant alkaloids/natural products, antitumor antibiotics, hormonal agents, and biological response modifiers.[6] Depending on the type and severity of the cancer, one or more of these categories of chemotherapy drugs will be administered to the patient. The more exposure the patient has to the drug, the more the health care providers will be exposed as well, thus increasing their overall genotoxic effects.[6]

Mechanisms of Chemotherapeutic Genotoxic Effects in Cells

Chemotherapeutics may cause genotoxic effects through a few different mechanisms. According to a study performed in 2017, chemotherapy induced toxicity to healthy cells occurs in one of two ways: directly or indirectly. Direct toxicity means that the chemotherapy induces the same methods of damage to healthy cells as it does to the cancer cells. However, indirect damage occurs from the release of cell-free chromatin (cfCh) from the dying cancer cells, which then damages the healthy cells by triggering the DNA damage response or causing inflammation (Figure 1).[7]

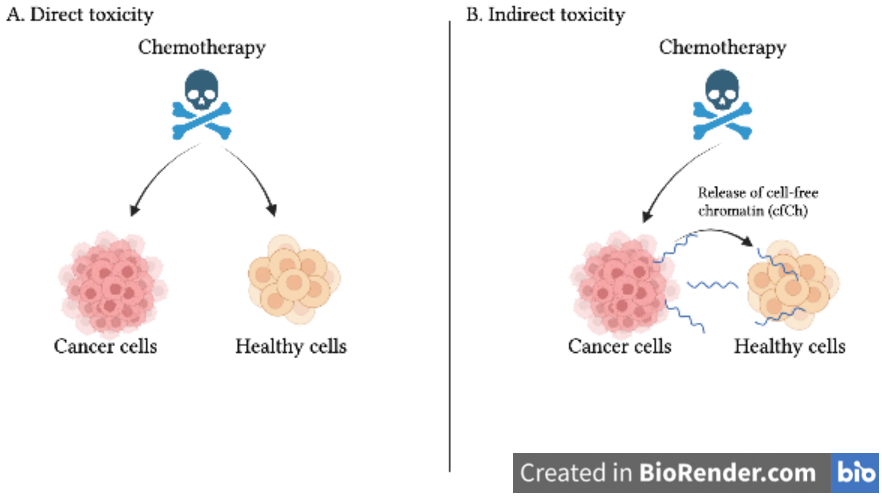

Figure 1. *Direct vs. Indirect Toxicity.*

Genotoxic Effects of Chemotherapy on Healthy Cells

A major genotoxic effect of chemotherapeutics is that they can cause a wide range of other cancers to arise in a patient. For example, cells within the bone marrow are vital in maintaining hematopoiesis after chemotherapy. However, it is known that these cells are damaged greatly by chemotherapeutic drugs and the mutations induce many types of bone marrow cancer. In 2017, a study was performed to determine the long-term effects of damage to these cells in patients specifically receiving the alkylating agent - cyclophosphamide - which can cause a reduction in healthy cells. Using the micronucleus and comet assays, it was determined that there was a significant increase in DNA damage and the numbers of abnormal cells increased. Genetic lesions also occurred, adding to the genetic instability and risk for future therapy-related malignancies.[8]

Myelodysplastic Syndromes and Acute Myeloid Leukemia are very common therapy-related malignancies. Over time, there has been a great increase in these malignancies since the high success rate of chemotherapeutic drugs has increased the drive to use the treatment and thus overall human exposure. “Therapy-related leukemias are a major problem in patients treated for Hodgkin's disease, non-Hodgkin's lymphoma, myeloma, polycythemia, breast cancer, ovarian carcinoma, or testicular carcinoma”.[9] Studies have found that the highest potential of developing a secondary leukemia comes from treatments using alkylating agents, nitrosoureas, and procarbazine. Despite the evident genotoxic risk, doctors often determine that the risk is worth it in order to treat the active cancer in patients.[9]

Genotoxic Effects on Healthcare Providers Administering Chemotherapy

One study, performed in 2007, assessed the genotoxicity caused to the nurses and doctors administering chemotherapy drugs. It was reported that "occupational exposure to anti-cancer drugs can represent a potential health risk to humans." Both the comet assay and micronucleus assay test were used in this study. They found both an increase in DNA damage in lymphocytes and an increase of micronuclei found in the nurses being exposed to the drugs.[10] As indicated by the results of this study, more stringent safety precautions are necessary to maintain the safety of those administering and handling these powerful drugs.

Prevention of the Genotoxic Effects of Chemotherapeutics

There have been many research initiatives focused on preventing healthy cell damage that occurs from chemotherapy treatment. The first study that was looked at was based on the hypothesis that DNAse is an enzyme that breaks down the hydrogen bonds that hold the two DNA strands together (Figure 2). The study suggests that "treatment with DNAse or other DNA neutralizing agent can prevent chemotherapy-induced toxicity in healthy cells".[12] The powerful enzymes have the ability to break these necessary bonds within the cancer cells. The "results suggest that toxicity of chemotherapy is caused to a large extent by cfCh released from dying cells and can be prevented by concurrent treatment with cfCh neutralizing/degrading agents".[7]

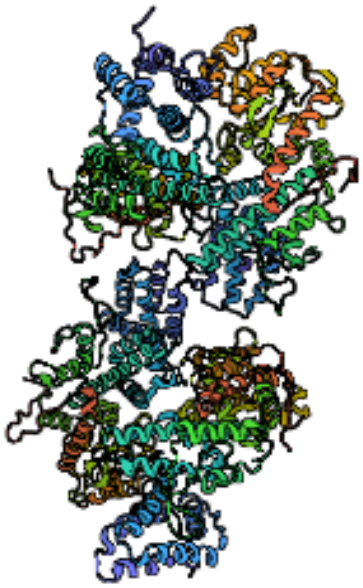

Figure 2. *Structure of a DNase Protein.*

An additional study performed by Changhan Lee in 2012 linked the effects of nutrition and chemotherapy-induced toxicity to healthy cells.[13] This study was based on the hypothesis that "normal cells and cancer cells differ in their ability to respond to fasting".[14] When a patient fasts, the normal cells are able to switch their metabolism by utilizing maintenance pathways; however, cancer cells are unable to perform this task. Therefore, by utilizing short-term fasting during chemotherapy treatment, the risk of toxicity can be lessened greatly by starving the cancer cells to death.[15] A pilot trial to this hypothesis was conducted, which produced promising results. The trial included thirty patients aged 30-74 years, who underwent a minimum of four chemotherapy cycles. The patients fasted intermittently for half of the chemotherapy cycle, making sure they still received enough nutrition during the non-fasting periods of intermittent fasting. For the second half of the cycle, patients maintained a normal diet. The results indicated that utilizing modified short-term fasting (mSTF) during treatment can both reduce the chemotherapy-induced toxicities and enhance the tolerance of chemotherapy (Figure 3).[16]

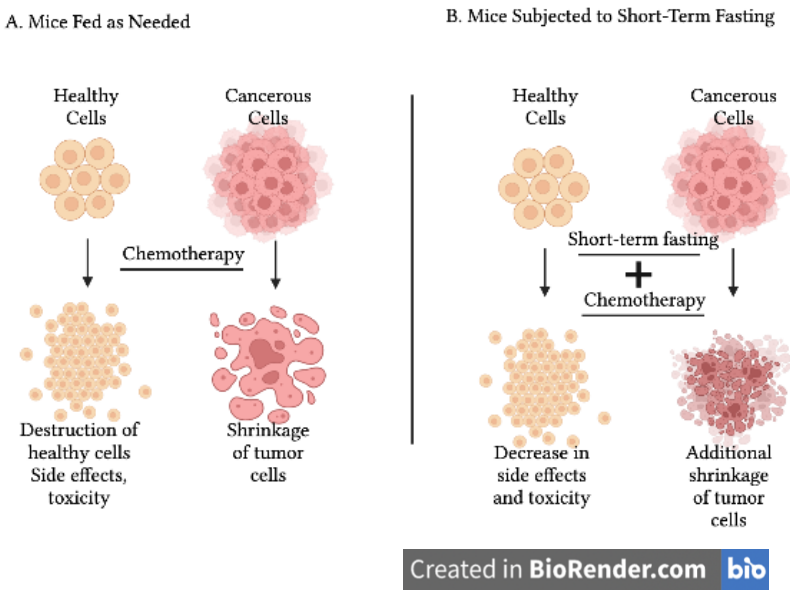

Figure 3. *Results of Short-Term Fasting while Receiving Chemotherapy Treatment.*

It is well known that diet plays a role in many aspects of human health. Interestingly, this extends to the influence of cancer development. The source for this is epigenetic reprogramming: a mechanism through which certain substances introduced through diet can influence gene expression, and can cause changes in cell proliferation and growth. Recent research has been conducted that explores the effects of fasting and ketogenic diets on chemotherapy outcomes. The main goal and determining success factors being reducing toxicity and improving the quality of life. [17]

With regard to fasting, through many studies it has been found that intermittent and short-term fasting during chemotherapy is safe and well-tolerated. Even more so, it may reduce fatigue, side

effects, and improve one's overall quality of life. A cohort study was conducted which found that fasting for around 48 hours is safe and feasible for cancer patients. It may reduce DNA damage in leukocytes. This study also demonstrated that fasting reduces DNA damage in mononucleated blood cells and may promote DNA recovery after chemotherapy. Another study was conducted that found that fasting may reduce nausea, vomiting, diarrhea, abdominal pain, and reported fewer side effects in patients who fasted for the entire duration of chemotherapy. Although promising results, further research is needed to confirm these findings.[17]

The use of ketogenic diets (KD's) in cancer treatment was also explored. KD's mimic the metabolic state of fasting and elevate ketone bodies above the reference range. Several studies have explored the feasibility and effects of KD's in cancer patients. A pilot study found that KD's were tolerable and safe in advanced metastatic tumors, and five patients completed a 3-month intervention period, reporting improved emotional functioning and less insomnia. Another study found that KD's were well-tolerated and potentially useful in controlling tumor growth in patients with recurrent glioblastoma. The effects of KD's in combination with bevacizumab treatment were also explored in a study with 53 patients observed compared to those treated with bevacizumab alone. Additionally, a randomized controlled trial found a significant improvement in adjusted physical function scores in women with ovarian or endometrial cancer who followed a KD for 12 weeks compared to those following the ACS diet.[17]

The study conducted by Caffa, Irene, and colleagues in 2015 aimed to investigate whether combining fasting with tyrosine kinase inhibitors (TKIs) could enhance the efficacy of cancer treatment. TKIs are drugs that inhibit the activity of enzymes called tyrosine kinases, which are often overactive in cancer cells and contribute to tumor growth and survival. While TKIs have shown promise in cancer treatment, they often have limited efficacy and can cause side effects.[18]

The researchers used mouse models and human cancer cell lines to demonstrate that fasting increased the sensitivity of cancer cells to TKIs, leading to enhanced tumor suppression. Specifically, they found that fasting activated the MAPK signaling pathway, which is involved in cell proliferation, differentiation, and survival. The activation of this pathway led to a decrease in the activity of the MAPK proteins, which are often overactive in cancer cells and contribute to tumor growth and survival. By inhibiting the MAPK signaling pathway, fasting enhances the anti-cancer effects of TKIs.[18]

The study concluded that combining fasting with TKIs could be a promising strategy for improving cancer treatment outcomes. Fasting has been shown to have several health benefits, including reducing inflammation, improving insulin sensitivity, and enhancing cellular stress resistance. By combining fasting with TKIs, it may be possible to enhance the anti-cancer effects

of the drugs while minimizing side effects. However, further research is needed to determine the optimal fasting regimen and to assess the safety and efficacy of this approach in clinical trials.[18]

Another study investigated the effects of a fasting mimicking diet (FMD) in combination with neoadjuvant chemotherapy (NAC) on breast cancer treatment. The study was a randomized phase 2 clinical trial conducted at multiple centers, and it involved women with early-stage breast cancer who were scheduled to undergo this type of chemotherapy.[19]

The study found that adding an FMD to NAC significantly increased the rate of pathologic complete response (pCR), which is a marker of treatment efficacy. The FMD group had a 36% pCR rate compared to 20% in the control group. The FMD also improved overall survival, disease-free survival, and quality of life in the patients.[19]

The study suggests that FMD can enhance the anti-cancer effects of chemotherapy and may have potential as an adjuvant therapy for breast cancer treatment. However, further research is needed to confirm these findings and to identify the optimal FMD regimen for breast cancer patients.[19]

Conclusion

As with many genotoxic drugs, both the pros and cons of the treatment and its risks must be evaluated in advance. Oftentimes with chemotherapy treatment, “although [carrying a] 52% risk of Grade III or worse toxicities, the mortality reduction benefit clearly outweighs the risks”.[20] Most studies conclude that the benefits of treatment, including the large increase in survival rate, outweigh the negative genotoxic risk the treatment poses. As science continues to advance and more studies/trials are conducted, the ability to eliminate many of these genotoxicities is possible, thereby ensuring a safer and more effective method of treatment for cancer patients around the world.

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Yeshiva University Journal of Medicine and Dentistry

Review

Benefits of the Novel Transcarotid Artery Revascularization (TCAR) Technique for the Treatment of Carotid Artery Stenosis

Devorah Chasen

Abstract

Cerebrovascular diseases consist of a group of cerebral blood vessel disorders which affect blood flow to the brain. Consequences of cerebrovascular disease include cell death and stroke due to cerebral hypoxia. Unfortunately, stroke is the fifth leading cause of death in the United States and the number one leading cause of disability. Carotid Artery Stenosis (CAS) is one of the most common cerebrovascular diseases that often leads to ischemic stroke. CAS is typically caused by the buildup of atherosclerotic plaque, which consists of fatty substances, cholesterol, cellular waste products, calcium, and fibrin, along the inner lining of the carotid artery. As the plaque grows, it protrudes into the lumen of the artery causing stenosis; subsequently, the plaque may rupture and promote the formation of a blood clot that further occludes the artery. Medical management is effective at controlling the effects of Carotid Artery Stenosis and preventing future strokes. However, in certain cases of severe stenosis, surgical intervention is often recommended. The Carotid Endarterectomy (CEA) was the standard surgical intervention for revascularization for almost seventy years. Until recently, no other intervention compared to it in its effectiveness and safety. However, with the introduction of the Transcarotid Arterial Revascularization (TCAR) technique in 2015, a new standard for interventional cardiology was born. Studies over the past seven years have shown promising results for this novel procedure, which combines the methods of traditional stenting with a blood flow reversal neuroprotection system to create a quick, efficient, and minimally invasive revascularization option. Future studies hope to further prove its effectiveness as it becomes a more mainstream procedure to treat CAS.

Introduction

Cerebrovascular disease is a group of disorders characterized by bleeding in or restricted blood flow to a part of the brain that causes temporary or permanent effects and involves one or more cerebral blood vessel.[1] It is essential that the heart pumps enough blood to the brain so that oxygen and important nutrients can also be transported to the brain and circulated throughout. When cerebral ischemia occurs, or blood flow to the brain is reduced or restricted, it puts the patient at an increased risk of developing a stroke.

Possible causes of ischemia to areas of the brain are due to vessel stenosis (narrowing), thrombosis (blood clot formation), embolism (foreign clot or debris blockage), or hemorrhage (rupture).[1] Carotid artery stenosis, also known as carotid artery disease, is one of the primary forms of cerebrovascular disease.[1] The carotid arteries, along with the vertebral arteries, are the main vessels that supply the brain with oxygen and essential nutrients.[2] Therefore, damage to the carotid arteries, such as stenosis, can cause cerebral hypoxia, or a decrease of oxygen supply to the brain, which leads to the death of the affected tissue and an ischemic stroke. Unfortunately, in the United States, strokes are the fifth leading cause of death, and about 3% of adults will experience a stroke in their lifetime.[3] Strokes are the leading cause of long-term disability, as those who suffer a stroke are often left with reduced mobility. Furthermore, one-third of people who suffer a stroke are left permanently disabled.

Carotid Artery Stenosis

Carotid artery stenosis is almost always caused by atherosclerosis, which is the buildup of fatty substances, cholesterol, cellular waste products, calcium, and fibrin into the plaque along the inner lining of the carotid artery, the intima layer. As seen in Figure 1, the carotid artery is made up of three layers: the intima, the smooth innermost layer; the media, the muscular middle layer; and the adventitia, the protective outer layer. As the buildup of plaque increases, the lumen of the artery narrows (stenosis), and the walls of the artery become thickened and stiff. Through various mechanisms, the atherosclerotic plaque can cause significant stenosis over time, which may lead to an ischemic stroke. Therefore, it is important to be proactive at managing risk factors even from a young age, which is when the atherosclerotic plaque begins building up.[4]

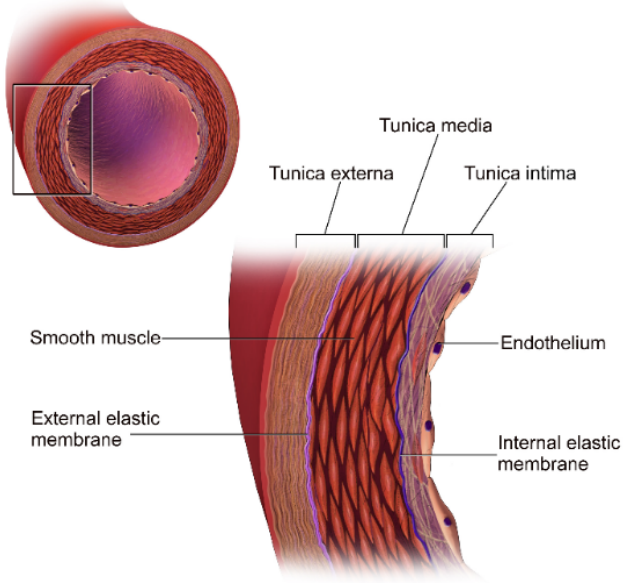

Figure 1. Layers of the Carotid Artery[19]

Mechanism of Stroke Due to Atherosclerosis

As a fibrous plaque grows, it accumulates more fatty lipids and cholesterol. Once the plaque has reached a certain size, it begins to protrude into the lumen of the artery. This causes partial occlusion of the vessel, or stenosis, and it may reduce the amount of blood that can flow through the artery.[5]

Thrombosis and embolization, which are other complications from atherosclerosis that result in blood clots, can occur and contribute to a carotid stenosis-related stroke. As the atherosclerotic plaque grows and becomes unstable , it also becomes increasingly likely to rupture.[6] When plaque ruptures, the cholesterol and collagen become exposed, and the body recognizes this occurrence as an injury. This injury stimulates the release of pro-coagulants and activates thrombosis or the clotting cascade to create a blood clot (Figure 2).[5] Occlusion of any portion of the carotid artery, whether from a thrombus or embolized thrombus, may result in cell death and subsequent ischemic stroke.

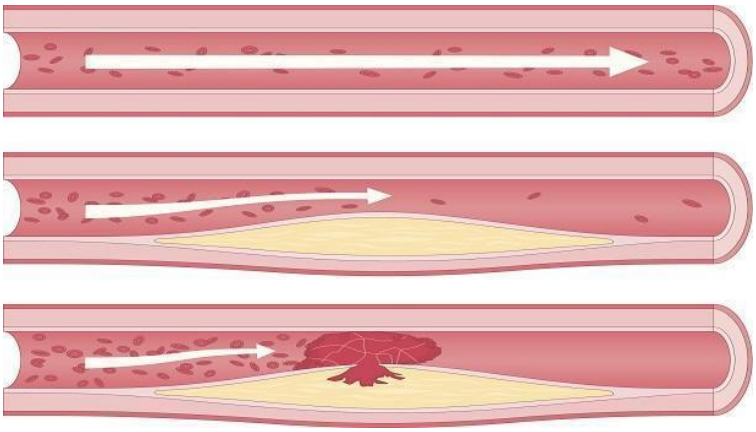

Figure 2. Blood Clot Formation[20]

Clinical Presentation of Carotid Artery Stenosis

Patients can have symptomatic or asymptomatic carotid artery stenosis. Symptomatic carotid artery disease is defined as neurologic symptoms that are sudden in onset and ipsilateral to significant carotid atherosclerosis. It also includes a TIA, characterized by focal neurological dysfunction or transient monocular blindness, a minor, non-disabling ischemic stroke).[7] In addition, in order to be considered "symptomatic," a patient must have had carotid symptoms within the past six months.[8] Management of symptomatic carotid artery stenosis is important because atherosclerotic plaques are usually embologic and pose an increased risk of causing another stroke.

Asymptomatic carotid artery stenosis refers to stenosis of the carotid artery without accompanying neurological symptoms or stroke. Many randomized and controlled studies have shown that there is only a small risk of stroke (1-2%) in asymptomatic cases of carotid stenosis

for mild (<50% stenosis) or moderate (50-69% stenosis). Because of these findings, medical management and longitudinal monitoring is typically the recommended course of treatment for patients with asymptomatic carotid stenosis. However, surgical intervention is sometimes recommended in cases of asymptomatic patients with a greater degree of stenosis (>80% stenosis).[9]

Risk Factors

The risk factors for carotid artery stenosis can be divided into two categories: non-modifiable and modifiable risk factors. Non-modifiable risk factors are those that cannot be controlled by a person, like age, sex, race and ethnicity, and family history. Modifiable risk factors are those that can be controlled by a person to minimize the risk associated with them and include hypertension, smoking, high cholesterol, hyperglycemia, diet, weight, alcohol consumption, and drug use. Oftentimes, nonmodifiable risk factors are linked to or exacerbated because of modifiable risk factors.[10]

Treatment

Many factors impact treatment options for carotid artery stenosis, and it is important to balance the risk of stroke vs. risk of intervention for the patient. Many forms of carotid artery stenosis can be managed with medical therapies so that the disease won't progress to the point of risking a stroke. Common medications given are antihypertensives to reduce blood pressure, statins to control LDL cholesterol levels, and antiplatelets to prevent clot formation. Carotid revascularization is also an option for certain individuals who could benefit from additional interventional help. These types of surgical interventions are oftentimes used for more extreme cases of stenosis but have been effective in preventing strokes or other symptoms of carotid artery stenosis.[11]

Surgical Intervention

There are three main surgical procedures to treat carotid artery stenosis that have been instituted over the past seventy years: the carotid endarterectomy (CEA), transfemoral carotid artery stenting (TFCAS), and transcarotid artery revascularization (TCAR).[12]

Carotid Endarterectomy (CEA)

The carotid endarterectomy (CEA) has been the gold standard for carotid revascularization procedures since the mid-1900s. Many randomized controlled trials show that this method is safe and effective at minimizing the risk of stroke in symptomatic patients with moderate to severe stenosis.[13] During the procedure, found in Figure 3, the surgeon performs an arteriotomy, in which he makes an incision in the neck and carotid artery at the carotid bifurcation or location of the plaque. The surgeon then removes the plaque, and the artery is repaired using sutures or a patch which widens the lumen of the artery. While this method is invasive, it produces promising results for those affected by moderate to severe symptomatic carotid artery stenosis.[12]

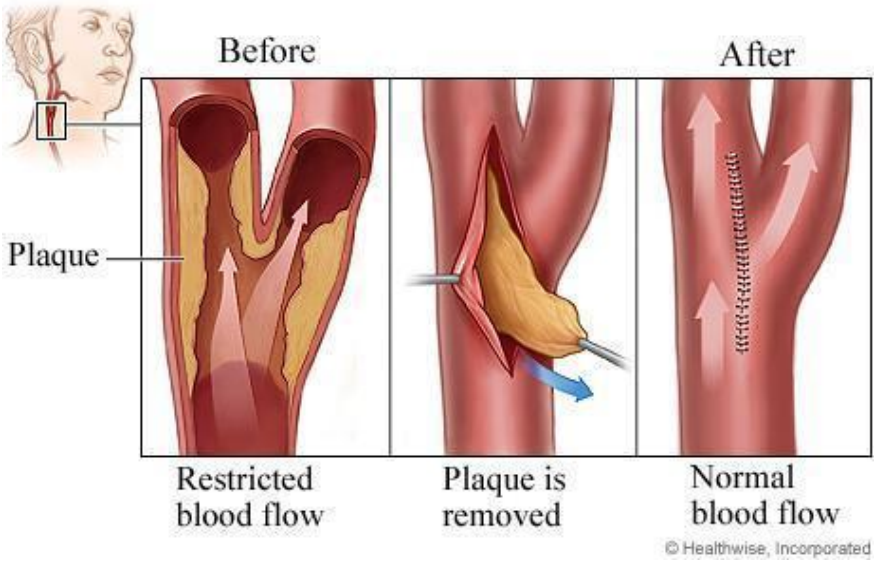

Figure 3. Carotid Endarterectomy (CEA) Procedure[21]

Transfemoral Carotid Artery Stenting (TFCAS)

The transfemoral carotid artery stenting (TFCAS) intervention was introduced in 1996 as an alternative, minimally invasive technique to the CEA procedure.[14] During the procedure, a sheath and catheter are placed in the femoral artery in the groin followed by a carotid stent. The stent is advanced until it reaches the carotid artery and then expanded. An angioplasty balloon is used to ensure the stent is positioned properly against the arterial wall and stabilizes the plaque. The surgeon will use an embolic protection device to filter and catch any debris that may break off during the procedure, and intraoperative imaging and contrast material is used to visualize the vessels.[14]

Many studies have been conducted to compare the outcomes of CEA vs. TFCAS. The Carotid Revascularization Endarterectomy Versus Stenting Trial (CREST) determined that composite outcomes (stroke/death/MI) are similar between the two procedures. Unfortunately, the CREST trial also found that the TFCAS method has an increased risk of 30-day perioperative stroke compared to the CEA, likely due to aortic arch manipulation resulting in embolization. In fact, a meta-analysis done in 2012 found that the 30-day risk of perioperative stroke or death was 8.2% for TFCAS vs. 5.0% for CEA.[14] Because the CEA is still the ideal method of choice for revascularization, the TFCAS is typically only used when the patient is at high risk for the CEA due to it being an open surgery.

Transcarotid Artery Revascularization (TCAR)

A novel stenting method was introduced in 2015 called the transcarotid artery revascularization (TCAR) procedure. It combines carotid bifurcation stenting with an advanced reversal of blood flow, so the risk of plaque embolization is minimized. In preparation for the procedure, patients are prescribed the DAPT (dual antiplatelet) regimen statins. During the procedure, the surgeon makes a small incision (2 to 4 cm) just above the collarbone ipsilateral to the atherosclerotic plaque. A sheath is placed inside the carotid artery as well as a neuroprotection system. This system provides CEA-like neuroprotection for the patient through high-rate temporary flow reversal; any blood flowing to the brain is reversed and redirected outside the body where it gets filtered through a device before it is returned to the body via a sheath in the femoral vein (Figure 4).

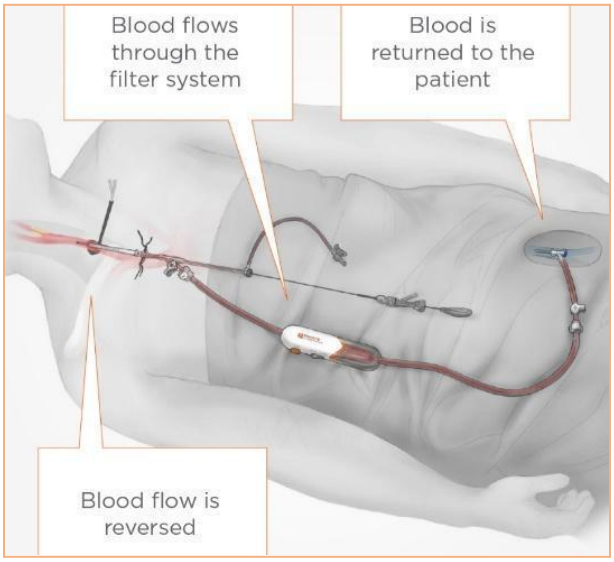

Figure 4. TCAR Neuroprotection System Providing Flow Reversal[22]

Once the flow reversal system is in place, a stent is placed inside the carotid artery to stabilize the plaque and prevent future stroke (Figure 5) and fluoroscopy is used to monitor the procedure.[15]

Figure 5. TCAR Stent Insertion[23]

The primary study conducted evaluating the TCAR procedure is the Safety and Efficacy Study for Reverse Flow Used During Carotid Artery Stenting Procedure (ROADSTER). The initial results of the TCAR procedure as described by the ROADSTER multicenter trial show that TCAR with ENROUTE neuroprotection system is a safe and effective method for preventing a stroke. The 30-day perioperative stroke rate was only 1.4% in surgical high-risk patients, which is lower than in patients who had the TFCAS procedure. Additionally, 1.4% of patients died, and 0.7% of patients suffered an MI, thus resulting in a 30-day stroke/death/MI rate of 3.5%.[16] The ROADSTER 2 trial had a 30-day stroke/death/MI rate of just 1.7%.[17] These low rates of stroke, death, and MI with the TCAR are most likely due to avoidance of aortic arch manipulation and the advanced neuroprotection system. With TFCAS, it is often difficult to avoid aortic arch manipulation which results in embolization and subsequent stroke, death, or MI. With the TCAR procedure, the flow reversal system also protects against embolization by plaque that breaks free during the procedure.[16]

The results of TCAR were also comparable to CEA results, as the Transcarotid Artery Revascularization Surveillance Project showed no significant differences in 30-day perioperative stroke or death outcomes between TCAR and CEA. In fact, the results for the TCAR procedure were obtained using a high-risk patient population, which shows the similar effectiveness of the TCAR compared to CEA. The main difference in outcomes between the TCAR and CEA was the significantly lower rates of cranial nerve damage with the TCAR procedure. This is because there is only one vulnerable cranial nerve that can be injured in the TCAR procedure vs. four in the CEA.[18] Additionally, positive results were obtained from surgeons with little to no experience in the TCAR procedure, which speaks to the safety of the procedure.[17] There is also reduced

operative time with the TCAR procedure, which is beneficial to both the surgeon and patient. The preliminary results of TCAR studies are promising because no other method to date has had comparative results in outcomes to CEA. Because of the introduction of the TCAR and emerging data on its effectiveness, it is likely that the TCAR will become the standard of care in the future for revascularization.[14]

Conclusion

Carotid artery stenosis and associated strokes have been of concern as they are one of the leading causes of death and disability worldwide. Fortunately, there are many types of treatments to manage carotid artery stenosis. Medical treatment and surgical intervention have shown promising results, and the introduction of the TCAR procedure is a significant contribution to the field of vascular surgery. The implementation of this new minimally invasive procedure has already benefited many patients and will hopefully continue to do so in the future.

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Yeshiva University Journal of Medicine and Dentistry

Review

Exercise as a Mechanism to Combat COVID-19 Related Cardiovascular Problems

David Marocco

Abstract

Symptoms and infection of COVID-19 virus have been observed to vary among carrier patients. While a majority of cases possess short term symptoms such as loss of taste, congestion, fatigue, etc, COVID-19 can cause long lasting symptoms beyond the infectious period. This is clinically known as post-COVID-syndrome (PCS). PCS can lead to cardiovascular complications, especially in individuals with pre-existing heart issues. Exercise is a well known combatant of cardiovascular disease and can be used to minimize the degree of the symptoms. Exercise also has the ability to prevent COVID-19 symptoms as a whole by strengthening the cardiovascular and immune system.

Introduction

In December of 2019, the COVID-19 pandemic, also known as SARS CoV-2, took the world by storm and began infecting millions of people. COVID-19 is a highly transmissible disease that infects the respiratory system with the ability to affect other major systems of the body.[1] Those who have been infected severely enough suffer long lasting symptoms, a phenomenon called post-COVID-syndrome. Depending on the severity of infection and the individual, long lasting effects vary from loss of taste and smell to chronic fatigue and headaches. There has also been a reported impact on the cardiovascular system of those who suffer from post-COVID-syndrome, including chest tightness, palpitations, syncope, dysautonomia, hypotension and other heart related illnesses.[2] While treatment for select issues is available, research shows that those involved in daily physical exercise reduce their likelihood of developing cardiovascular issues when infected with COVID-19, as well as reduce the severity of their symptoms if they do occur.[2]

post-COVID-syndrome

According to the United Kingdom National Health Service, post-COVID-syndrome (PCS) is defined as "unexplained, persisting signs or symptoms over 12 weeks, developed during or after the COVID-19 infection."[2] The symptoms for post-COVID-syndrome are numerous and wide-ranging. Studies have shown that people with PCS continue to suffer from a multitude of ailments including severe body ashes, migraines, and chronic fatigue even months after being infected.[3] An online survey reported more than 200 lasting symptoms among people who have experienced PCS.[2]

Post-COVID syndrome is a relatively common occurrence. A study that began in April 2020 by the UK's Office for National Statistics showed that 13.7% of people who tested positive for COVID-19 between April 26, 2020 and March 6, 2021 continued to show symptoms after 12 weeks.[2] This signifies that although most people overcome the effects of COVID-19, a significant number of people do not. The numbers are staggering when considering those who were hospitalized with COVID. A revealing study indicated that up to 87% of people who were once hospitalized with COVID-19 show long lasting symptoms.[4]

Unfortunately, there are many questions yet to be answered regarding this condition regarding the causes and possible treatments. Medical professionals are still trying to determine its cause and why matters such as severity of infection do not directly correlate to the severity of the syndrome. For example, non-hospitalized COVID victims have reported lasting effects. In a study published in September 2021 on non-hospitalized COVID-19 victims, 30% reported fatigue and 37% reported cognitive impairment after infection.[4] Post-COVID-syndrome has also been shown to be a severe risk on those with cardiovascular issues and has the ability to cause the development of heart conditions as well.

Immune System Response to Heart Infection

In order to fight infection, the immune system must act quickly in order to minimize both direct and indirect damage to the heart. The beginning stage of myocarditis begins with viral replication and direct damage to the muscular tissue of the heart. When this occurs, the virus is recognized by host factors called pattern recognition receptors (PRRs). These factors stimulate the production of antiviral proteins that initiate the immune response and heart tissue repair. Firstly, the innate immune system attacks with neutrophils, natural killer cells and macrophages. This is followed later on by the response of the adaptive immune system. The second stage of myocarditis consists of immune-mediated damage. This happens as a result of "cardio modulatory cytokines by immune cells, from the release of cytotoxic molecules that damage healthy cells, or from the presence of cardiac autoantibodies."[5] Depending on the severity of infection as well as the individual, the disease may be resolved or continue into a more severe, chronic phase.

COVID-19 and Myocardial Infarction

COVID-19 has the ability to disrupt functions of the body. Among the functions interrupted, the cardiovascular system stands out because of COVID-19's harmful effects. Firstly, it is important to understand how COVID-19 accesses the cells. The ACE2 protein is a cardioprotective transmembrane protein which is found in great amounts in the myocardial tissue. The COVID-19 virus attaches to the ACE2 receptor to gain access to the cell, and downregulates its expression.[6] A study performed on mice infected with the human strain of SARS-CoV was conducted to examine ACE2 protein expression in the hearts of those infected. The study found that the

presence of SARS-CoV in the heart was associated with the reduction of ACE2 protein expression.[7]

Due to COVID-19's ability to attack the heart, many people continue to suffer from COVID-related heart issues. This has the ability to complicate pre-existing diseases and oftentimes can worsen them. Patients with Congestive Heart Failure (CHF) are particularly vulnerable to complications. CHF occurs when the heart cannot properly pump blood to the body due to weaknesses in the heart muscle.[8] In some cases, there is an excess pooling of blood in certain parts of the body such as the legs, belly or even the lungs.[9] As of 2017, approximately 5.7 million people in the United States were reported to have CHF with estimates of the US reaching 8 million by 2030.[8] There is no singular cause for CHF, but those who smoke and drink regularly, or do not maintain a healthy diet and weight are more likely to develop CHF.[9]

With regard to COVID-19 and CHF, mortality rates and hospitalization time for people with both were observed to be higher than those without CHF. During a study conducted in 2021 at Mount Sinai Hospital systems in New York, a total of 6,439 patients were admitted to the hospital due to COVID-19, 422 of them with a history of CHF. Compared to the COVID-19 patients without a history of CHF, those with a history had higher systolic blood pressure and lower oxygen saturation. They also were at a greater risk for mechanical ventilation and on average spent two more days in the hospital than those without a history of CHF.[10] This indicates that COVID-19 has the ability to complicate pre-existing heart conditions and put some people at a greater risk of developing lasting effects.

COVID-19 has the ability to attack the heart, causing or contributing to heart related problems. But the effects of COVID-19 are not just related to CHF. There is also a direct relationship between people with cardiac diseases and mortality and morbidity (2). In order to avoid these issues, it is very important for people to put themselves out of risk of heart related diseases. While screening for diseases is a start, it may come too late in certain cases. A well studied aid to preventing heart disease, however, is exercise. Unlike screening, exercise can never come too late and has immediate benefits. Exercise can protect against cardiovascular disease as well as the effects of COVID-19.

Exercise Improves Cardiovascular Health

One of the most universally accepted ideas is that exercise has tremendous health benefits and can improve many aspects of physical health, including the cardiovascular system (Figure 1). Numerous studies have shown that daily physical exercise contributes to a healthier heart.[2] However, even with the evidence from these studies, many people are unable to start doing such exercise. According to the Center for Disease Control and Prevention (CDC), less than 25% of the adult population in the United States perform daily exercise.[2] This means that people are putting themselves at a higher risk of cardiovascular issues because they are not strengthening

their heart, regardless of whether or not they contract COVID-19. If they do contract the disease, there is an even higher risk of developing issues because they might have a weaker cardiovascular system due to their physical inactivity .

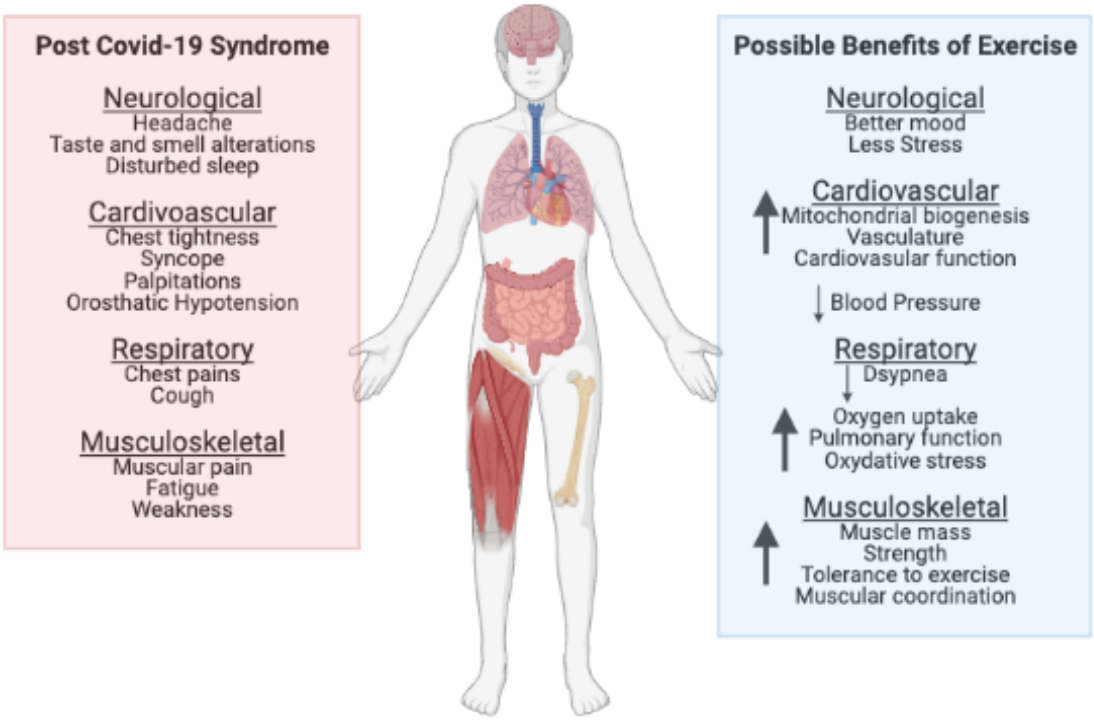

Figure 1. *Possible beneficial outcomes of exercising on common post-COVID-19 syndrome symptoms. (Adapted from reference 2)*

The cardiovascular system is greatly improved by physical exercise. When blood travels through the body, it releases oxygen that is needed for our body to function. Cardiovascular exercise improves the heart's ability to pump the blood around and thereby supply oxygen to the rest of our body. When one runs, the body requires more energy in order to move, so the heart must work harder and blood is more quickly pumped through the body. If blood is flowing through the body more quickly, then supply of oxygen to the muscles increases, improving their abilities.[11] Exercise trains the body to pump blood faster and increase production and efficiency.

Those with CHF also have a reported lower peak oxygen consumption (V02 Max). Peak oxygen consumption is defined as the "product of cardiac output (CO) and arteriovenous oxygen (AVO2) difference."[11] They are not receiving adequate oxygen amounts, and their hearts are not functioning totally properly as a result of the lower oxygen levels caused by the heart disease. Exercise can improve the V02 max though. In a study consisting of over 2,102 individuals aged 60 and older, aerobic exercise, or "cardio", caused a 16.3% increase in peak oxygen consumption.[11] V02 max is important because it is an indicator of how well an individual's heart

is pushing blood around the body.[11] Therefore, one with a higher peak oxygen consumption has a higher functioning heart.

Many other clinical trials have shown that moderate daily exercise can improve the cardiovascular system. A study conducted over a 4 month period was done in order to determine the effects of inputting exercise in the daily routine of individuals who have metabolic syndrome. Metabolic syndrome is defined as a cluster of conditions that increases the risk of heart disease.[11] The study reported that after the 4 months, the individuals "had reduced adiposity, decreased systolic, diastolic and mean arterial blood pressure, and lower total and low-density lipoprotein (LDL) cholesterol lipid profiles compared to the control group."[11] Participants of the study were found to be at a lesser risk for heart disease due to physical activity.

Obesity is another factor that can increase the risk of heart disease. Obese individuals require more blood to supply their bodies with oxygen. This results in an increase in blood pressure as the body works harder to move more blood around the body.[11] Exercise decreases body fat, lowering the risk of heart diseases. Even walking a certain amount of distance per day can significantly increase cardiorespiratory fitness in obese individuals and ultimately lower the risk of cardiovascular disease.[11]

Exercise even has benefits for individuals who have already been diagnosed with a disease. Even though they can no longer prevent the disease from occurring, it does not mean exercise will not improve their health. Exercise can be used as a therapeutic treatment in those who have cardiovascular disease and can significantly decrease mortality rate amongst these people while having the ability to improve their conditions.[12] An example of this can be found in patients with CHF who performed 4 weeks of high intensity interval training (HIIT). The results of the study showed that these individuals had an improved peak oxygen consumption and reduced diastolic dysfunction.[11]

There is also a direct correlation between exercise and the immunological response. Factors such as intensity and frequency of exercise have impacts as well. For example, muscle contraction promotes the release of both pro and antiinflammatory cytokines at levels that vary based on intensity and duration of the movement (Figure 1).[13] The release of cytokines recruit neutrophils to the site, which engulf and destroy infecting pathogens. Physical activity also increases the concentration of leukocytes circulating in the blood. This concentration remains at its peak 30-120 minutes after exercise and can persist for almost 24 hours.[13]

Conclusion

Evidently, COVID-19 can cause complications in the cardiovascular system and lead to worsening symptoms of post-COVID-syndrome. Therefore, it is important for people to exercise to improve their cardiovascular health, as well as boost their immune response to infection.

Exercise can prevent heart related issues, making it important for people to exercise so that COVID-19 will have a limited effect on cardiovascular diseases. For individuals already suffering from heart disease, it is important for them to exercise as a mechanism to improve their state of health. This will reduce the likelihood of post-COVID-syndrome.

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Yeshiva University Journal of Medicine and Dentistry

Documentary

Stop it Before it Starts: Oral Health Care Disparities

Renee Benjamini

Abstract

Tooth decay, or dental caries, can begin in early childhood. Though, it consistently affects people of all ages across the world. Tooth decay is caused by bacteria in the oral cavity that can create a biofilm conducive to its growth. The bacteria produces compounds which will continue to eat away at tooth material further into the root until pain and infection begin — unless stopped by treatment. Unfortunately, treatment is often inaccessible. Oral health status is affected by many factors which result in global disparities. A deteriorated oral health status is more common among individuals with risk factors, like a low socio-demographic status. The cost of care for the rampant amount of untreated caries is too high for many low-income countries to cover. Therefore, it falls upon supplemental organizations to close the gap in global oral health care through education and treatment.

Introduction

The lifelong struggle with oral health often begins in early childhood and will continue to progress throughout adulthood. Oral health care is essential to prevent serious issues. When the oral cavity is not cared for properly there are a slew of events that take place ultimately leading to pain, damage, and increased overall health risks. Bacteria present in the oral cavity utilizes the food an individual eats and produces lactic acids. The acid slowly destroys the layers of the teeth causing more advanced issues unless it is identified and treated at the onset.

Tooth Decay

The most prominent dental diseases are tooth decay (also known as dental caries) and gum disease (also known as periodontal disease) — tooth decay is more widespread. The process of tooth decay occurs in a consistent manner. The bacteria, *Streptococcus mutans,* is present nearly everywhere, consequently within the mouth. As the bacteria proliferates it will form plaques which create a sticky film on the teeth. The biofilm provides a favorable environment for the bacteria. Tooth decay begins as a result of lactic acids on the enamel which are byproducts of the bacteria breaking down sugars found in the foods an individual eats. The lactic acid causes demineralization, or the loss of calcium phosphate, from the enamel. This causes the tooth to soften and eventually collapse — forming a cavity.

If a cavity is identified during the early stages — while it is still confined to the enamel — decay can be halted by a filling. However, when left untreated, it will continue to “eat through” the dentin and eventually the pulp (Figure 1). indicates the morphology of a tooth. Once the bacteria

has invaded the pulp the pain and infection cannot be treated through a filling. Rather it will require a root canal treatment or often an extraction.[1]

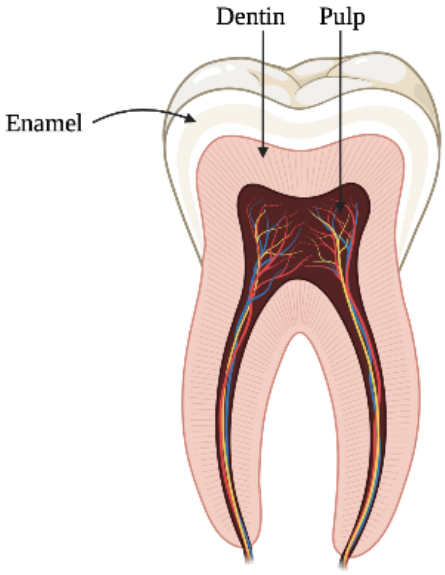

Figure 1. *Tooth morphology*

Disparities in Oral Health

Health Literacy is defined as "the degree to which individuals have the capacity to obtain, process, and understand basic health information and services needed to make appropriate health decisions."[2] According to a study done, the individuals with lowest oral health literacy were the poor, low levels of education, minorities, and the elderly.[3] Numerous studies and meta-analyses have been done to assess the disparities in oral health, their causes, and directives to mitigate them.

There is a strong correlation between low Oral Health Literacy and high risk for oral diseases and their associated problems.[4] Unfortunately, individuals with low health literacy are less likely to utilize preventative measures and instead seek emergency care resulting in more expensive and invasive procedures.[3] Both on the global and national levels there are inequalities in oral health.

Within the United States low income is directly correlated with a higher occurrence of untreated caries by factors up to three times those of individuals with higher incomes, across all ages. 17% of children between the ages of 2-5 in low income households suffer from untreated cavities in their primary teeth. This is three times that of children from higher income homes in the United

States. The disparities are not solely socioeconomic, level of education similarly plays a factor. Between the ages 20-64, adults with less than a highschool education are nearly 3 times as likely to have untreated cavities as those who attended a college.[5] It is apparent that individuals are less likely to seek care due to lack of proper education about oral health care and lack of access due to affordability.

Globally the problem is very similar to that in the United States. A study was done to assess the burden, trends, and inequalities of untreated caries in both permanent and primary teeth between the years 1990 to 2019 at many different levels, utilizing analytic methods. Untreated caries were found to be the most widespread health condition in 2019. It was similarly the leading disease in children between 0 and 14 years of age. The issue spans across the world, and was even found to be the most prevalent disease in 5 of the 7 named Global Burden of Disease super-regions.[6]

Through global analysis, the attribution to the burden of untreated caries in permanent teeth by sociodemographic inequality was assessed. The results showed that socioeconomically deprived people showed higher caries prevalence, and more commonly within developed countries. It was found that within the age range of 5 to 9 years, there was the largest percent of untreated caries in permanent teeth attributed to inequality. Additionally, countries with a lower socio-demographic index composed a higher percentage of untreated caries in deciduous teeth. Unfortunately, the study indicated that burden has maintained relative stability for decades.[6] According to the WHO oral diseases affect nearly 3.5 billion people globally, and 3 out of 4 are from middle-income countries.

Reducing Disparities

A study was done with the purpose of assessing the effectiveness of childhood education on oral health knowledge. Since oral health literacy is correlated with oral health status, oral health education is a step towards an effective preventative system. India was chosen as the population of interest as a majority of the population is poor and do not have access to sufficient oral health care.[7] In the study, 200 school-going children between 12-16 years answered a questionnaire about oral hygiene and proper care. Then, the students were given an in-depth course for the purpose of education. Afterwards, they were tested again. The new test scores, when compared to those before the intervention, indicated that there was a 60.4% increase in oral health care knowledge. For example, only around 50% of the participants knew that brushing twice daily was essential for maintenance and caries prevention, and only 27% knew the importance of routine dental check-ups. The studies showed that there was a general lack of knowledge of oral hygiene. However, through effective education the children may be able to prevent diseases and perhaps see the signs to identify them before their oral health passes the point of restoration. In this study, prior to implementation of education the students got most questions wrong. After implementation, barely any students got the answers wrong, indicating that the students were well educated on oral health after the seminar. The conclusion is a call to action for proper oral

health education beginning in childhood in order to diminish the struggle of high-risk individuals.[8]

In Amsterdam, the Netherlands, a group of researchers constructed a study that helps better understand families and professionals' perceptions of the inequality in oral health care and its causes. As of 2020, nearly 29% of children in Amsterdam did not visit the dentist. The researchers strongly believe that implementing oral health intervention programs will help reduce the inequality. This study is intended to lead to the development of an effective intervention program. In order to do so, professionals from a variety of dental and non-dental fields working in disadvantaged neighborhoods in Amsterdam, the Netherlands were interviewed regarding the oral health inequality and its primary causes. Since these individuals are involved in the lives of vulnerable families more directly, their insights were requested for this study. Some of the most commonly suggested causes were: unhealthy diet, children's non-compliance, and low oral health literacy in parents. With these results, it was suggested that the most crucial change is to implement a family-centered education program. It is equally important that family, teachers, community, as well as dental professionals, and non-dental professionals create a pro-oral health care environment. Increasing child and parent oral health knowledge in proper dental practices should be utilized as a preventative measure.[9]

The American Dental Association specifically notes that Early Childhood Caries are a serious, but preventable, disease that affects much of the population — specifically at-risk communities. Their primary method suggested to prevent early onset of a lifelong oral health struggle is through educating parents and expecting parents on oral health care and the importance of establishing a Dental Home before the age of one. Additionally, they assert that caries detection, classification, and prevention/risk management methods can reduce the patient's likelihood of more advanced diseases.[10]

Daily brushing and flossing is effective at preventing the accumulation of plaques in the most common areas.[1] Consequently, brushing and flossing prevents caries. A study over a 28 day period was done that evaluated whether brushing daily (compared to brushing less often) led to decreased plaque build-up. The study showed that brushing more frequently resulted in less plaque, calculus, and even reduced the severity of pre-existing gingivitis.[11] Additionally, fluoride can help the tooth resist the loss of calcium phosphate. Fluoride is often supplemented through toothpaste, tap water, rinse, or it can be professionally applied. Fluoride is effective because it is able to replace hydroxyapatite, found in tooth enamel, with fluorapatite — a harder compound.[1]

Some researchers have identified a flaw in modern dentistry. Namely that there is too much focus on treatment and technology, rather than utilizing the knowledge about prevention. For example, there are identifiable risk factors like diet, socioeconomic status, education, environment, and even biological factors that increase the risk of dental caries in an individual. Given these risk

factors, the first step should be prevention by mitigating them so that the caries are less likely to form at all.[12] Global education about oral health care preventative methods is essential in decreasing the burden of disease of untreated dental caries.[6] Though, it must be partnered with access to proper care and items like toothbrushes, floss, and fluoride.

Obstacle to Oral Health Equality

While the path to equality appears clear, providing education and care for the entire population may be an insurmountable obstacle. An analysis of the costs necessary to treat existing and future caries in the child population alone in developing countries was done. The calculations were executed using the WHO dental databases and spreadsheet calculations of costs. The results concluded that since 90% of dental caries are untreated in Third World Countries, the cost of treatment would be between $1,618 and $3,513 per 1,000 children between the ages of 6 - 18 years of age. This is found to be more than the resources allocated towards oral health care plans in 15 to 29 low-income countries. As such, it is not within the financial abilities of low-income countries to treat the dental caries in children.[13] While an option may be to redesign the finances of these countries, oral health care is often neglected as a priority leaving much of the population in consistent pain. Hence, it is essential to stop it before it starts. Caries prevention must be a priority if the cost of treatment is too high. Since fewer people would suffer from oral health diseases, investing in caries prevention systems would be a preferable solution financially to governments and to the general population.

Nonprofit Organizations

As concluded by many studies, it is imperative that there is education and access to oral health care provided to all. Specifically, in low-income homes and those with lower levels of education. This will allow a decrease in the frequency of emergent dental issues and oral health pain among neglected communities. In order to implement this, numerous organizations have been founded. Some provide treatment by sending out dentists and supplies and even construct tent clinics. Others provide education through free seminars and even distribute the essentials.

A Broad Smile Foundation

Many nonprofit organizations have been founded in order to help provide the necessary care across the world where there is improper access, education, and funding. However, in order for those organizations to successfully close the gap in oral health care it requires the assistance of donors and volunteers. A Broad Smile Foundation, one such organization, was founded with the mission to create change across the world through donations, supplies, and missions to provide care and education to communities without access. They do this through many outlets and the help of students and professionals. A Broad Smile Foundation organizes drives at middle and highschools to simultaneously gather oral health care essential items and educate about the importance of oral health hygiene. Additionally, they fundraise for missions in underprivileged communities to cover the costs of care delivered by volunteer dental professionals. They also

have begun teaching seminars that guide and educate about oral health care. Prevention through education is an effective method, as concluded by many studies. Individuals who do not have the capability of providing physical dental care, do have the ability to work on the prevention side. Oral health literacy among the entire population is a first step in abolishing the disparities in oral health status.

Conclusion

From the moment a tooth erupts, the risk of disease emerges. Early childhood caries affect so many children. The risks increase depending on many factors including diet, self care, oral hygiene, socioeconomic status, education, ethnicity, race, age, and more.[14] Many of the disparities in risk level can be prevented by education and access to care. It is imperative that people unify to provide this care and tame one of the most rampant global diseases.

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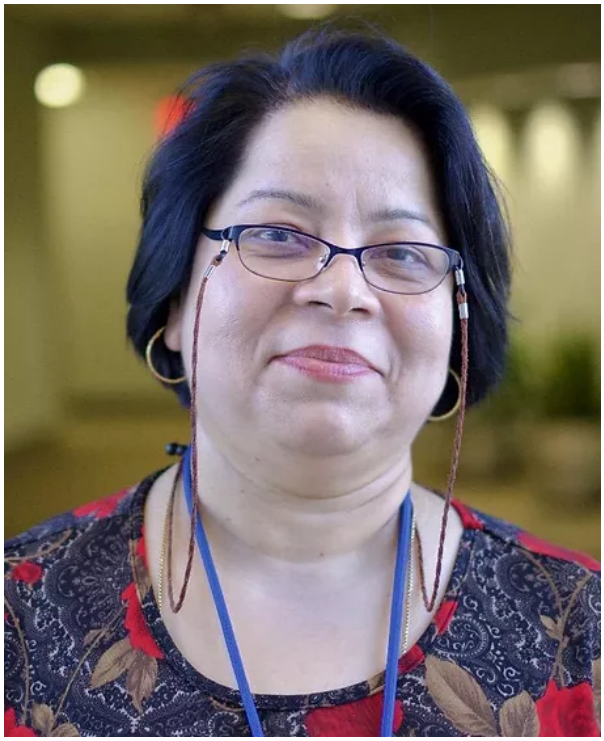

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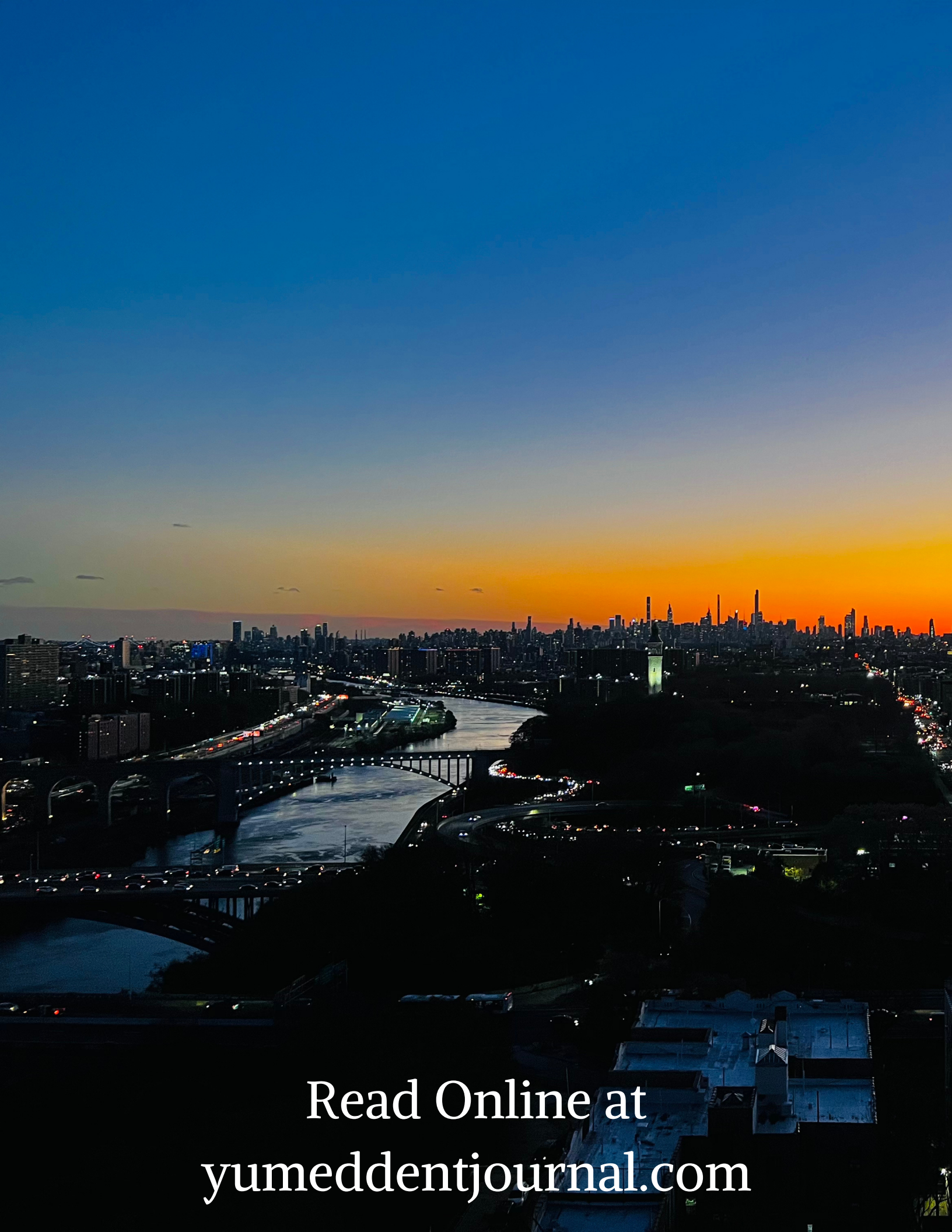
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