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RESEARCH ARTICLE

Exploring the Impact of Music Frequency and Decibels on the Viability of Neuroblastoma Cancer Cells

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ABSTRACT: This study examines the impact of music frequency on the viability of neuroblastoma cancer cells (SHSY5Y). SHSY5Y cells were exposed to music at varying frequencies and decibel levels, with "River Flows in You" by Yiruma (41 dB), "Brandenburg Concerto No.3" by Bach (62 dB), and "This is What it Feels Like" by Armin Van Buuren (86 dB) serving as the experimental stimuli. The cells' responses were analyzed after 24 and 48 hours of exposure, with cell viability assessed through automated cell counting devices using Acdine Orange and Propidium Iodide staining. Our findings show decreased viability of cancer cells exposed to high-frequency music. This result suggests a potential for high-frequency music to induce cancer cell death for cancer treatment. Conversely, low-frequency music increased the number of live cancer cells in the 2D culture model, indicating a music frequency effect on cell viability. These results contribute to the emerging field of bioacoustic oncology. Also, the frequency and intensity of the music could be leveraged for therapeutic purposes. This can offer a novel approach to cancer treatment with reduced side effects compared to traditional methods such as chemotherapy and radiotherapy.

KEYWORDS: Biomedical and Health Sciences; Cell, Organ, and Systems Physiology; Music; Brain Cancer; Cell Death.

Introduction

Brain cancer, with a survival rate just above 30%, stands as the leading cause of death worldwide, claiming the lives of over 250,000 individuals annually. The onset of brain cancer originates from a genetic mutation that enables normal cells to continue growing without responding to the signals to halt, which healthy cells typically respond to. Consequently, cancer cells multiply out of control, forming tumors as cell accumulation progresses. As the tumors advance, the grade of brain cancer escalates from Grades 1 and 2 to Grades 3 and 4.[1]

The brain tumor cell line of this experiment, *SHSY5Y*, is a neuroblastoma cancer cell that develops from immature neurons found in various regions of the body, most commonly the adrenal glands in the kidney, as well as other areas in the abdomen, chest, neck and near the spine. Neuroblastoma begins from neuroblasts, which are immature nerve cells in the fetus. These neuroblasts eventually differentiate into the adrenal glands' nerve cells as the fetus matures.[2]

Brain cancer patients typically encounter physical challenges like headaches, seizures, nausea, memory issues, and impairments in vision or speech. Chemotherapy and radiotherapy are the prevailing medical treatments to cure brain cancer, yet they bring side effects such as hair loss, hearing loss, vomiting, and anxiety.[3]

Music, a universally appreciated art form, has long been embraced as a therapy method capable of evoking emotional responses and changing human behavior. Yet, the mechanism behind these effects is still unidentified. Plato hypothesized that music played in different modes could elicit distinct emotions, and as a generality, most people agreed with his claim.[4] Nonetheless, several studies conducted since the beginning of the century suggest that the human brain's response to music may not be exclusively due to emotions. It has been discovered that many cells, apart from auditory hair cells, also directly respond to auditory sound, suggesting the effect of specific sound wavelengths on cells. Previous research has demonstrated that sound wave stimulation significantly changes the protein structure of Tobacco cells: an increase in the production of α helix and a decrease in β sheet.[5]

Music frequency, measured in hertz, refers to the speed of the vibration, which is the number of cycles of a sound wave that occur in one second. Decibel, on the other hand, is a unit that measures the pressure of the sound, in other words, the loudness of a sound. In the case of sound, increased frequency means shortened wavelengths and sharper pitch; therefore, the sound gets sharper, while increased decibel means higher intensity of a sound, resulting in louder sound.[6] The relationship between frequency and decibel is that the loudness of a sound can vary depending on the frequency of the sound. This is because the human ear is sensitive to different frequencies at the same sound intensity level.

A recent study has revealed that a university has developed a *Histotripsy* technique wherein sound waves physically obliterate tumor cells by disintegrating the cell wall cloak in mice.[7] Dr. Clifford Cho, from the University of Michigan, asserts that histotripsy causes cancer cells to undergo a unique cell death pathway that differs from other methods. Normally, when cancer cells are subjected to chemotherapy or radiotherapy, the tumor antigens–proteins concealed within the cancer cells' cell walls– are destroyed.[8] However, sound waves destroy the cells by rupturing their cell walls, releasing tumor antigens that trigger the body's defense system. This development can potentially offer medical professionals a treatment alternative

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for cancer patients devoid of the side effects associated with chemotherapy and radiotherapy.[9]

Overall, this research aimed to investigate the relationship between music with different frequencies and the viability of cancer cells. We hypothesized that music with higher frequency and decibels may induce cancer cell death more effectively, as shorter and sharper soundwaves may be more effective in destroying the cell walls and triggering cell death than music with lower frequency and decibels.

Methods

Cell culture and maintenance:

The SHSY5Y neuroblastoma cell line was purchased from the Korea Cell Line Bank. SHSY5Y cells were maintained in RPMI1640 cell culture media supplemented with 10% FBS and 1% penicillin and streptomycin. The cells were maintained healthy and subcultured every two to three days.

Music treatment on 2D and 3D cultured cancer cells:

SHSY5Y cells were prepared in 6 well culture plates and evenly distributed to test the effect of music on cancer cell viability. In addition to a negative control without music-exposed samples, the cells were then incubated with different types of music played at varying average decibel levels, as indicated in Figure 1. The cells were placed inside a 5% CO_2 incubator at 37 °C and incubated for 24 and 48 hours. Also, 3D cultured cancer cells were prepared using the hanging drop method.

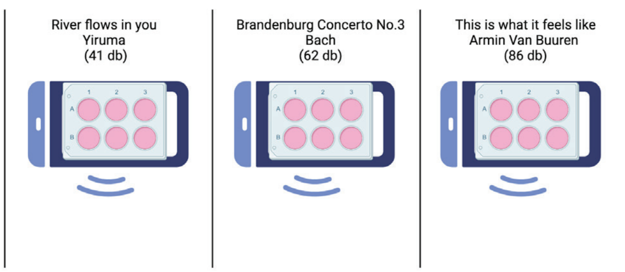

Figure 1: A schematic illustration of the cell culture method with three different types of music played at different average decibel levels on the bottom of the six-well plates cultured with SHSY5Y cells. "River Flows in You" by Yiruma (41 dB), "Brandenburg Concerto No.3" by Bach (62 dB), and "This is What It Feels Like" by Armin Van Buuren (86 dB) were played at the bottom of the culture plate using the iPhone. This illustrates the final music treatment setting for this experiment.

Cell viability analysis using automated cell counter device:

After the music was applied for 48 hours, the cells were detached from the cell culture plate using a Typsin-EDTA cell dissociation solution. Then, the cells were mixed with the cell-staining solution of Acridine Orange and Propidium Iodide. After the stained cells were applied on the cell slide, the viability of the cells was quantified using a LUNA-FL cell counting device.

Result and Discussion

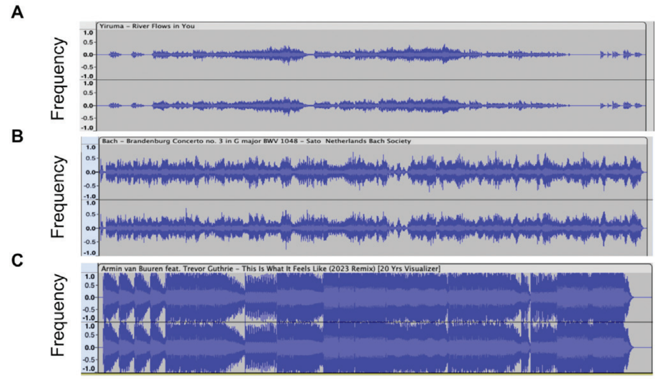

Figure 2: The frequency spectra of the compositions used in the study were obtained by the software Audacity version 3.4.2 for Mac. (A) The river flows in you by Yiruma. (B) Brandenburg Concerto No.3 by Bach. (A) River flows in you by Yiruma (B) Brandenburg Concerto No.3 by Bach (C) This is what it feels like by Armin Van Buuren. The results indicate differences in sound frequency among the three types of music.

To determine the music frequency of all three pieces of music, the files were opened in Audacity version 3.4.2 software for Mac (Figure 2). By observing the blue wavelengths that represent the music frequency, it is verified that each of the music falls under a different range of sound frequency. Music A was classified as low-frequency music as the vertical scale showed a waveform of approximately -0.3 to 0.3. Music B was the middle-frequency music, as the sound frequency was between -0.5 and 0.5, and music C can be identified as the high-frequency music, as the waveform was between -1.0 and 1.0. These distinctions in frequency were crucial for the study's design, as they allowed this study to assess the impact of different acoustic environments on the structural integrity of tumor cultures. The apparent differences in the sound frequencies of the selected music pieces provided a valid basis for comparing their effects.

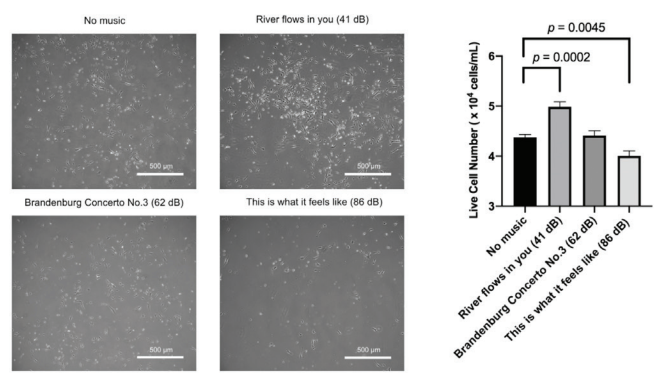

Figure 3: Low-decibel music (River Flows in You) significantly increased the number of live cancer cells. Still, high-decibel music (This is What it Feels Like) significantly decreased the number of live cancer cells. This result indicates that low-decibel music may enhance the cell proliferation of brain cancer cells, while higher decibel may induce cell death of brain cancer cells.

Figure 3 aims to show the effect of music treatment on brain cancer cell morphology and cell viability, precisely the number of live cells. Cell morphology was examined using the bright-field imaging technique from an inverted microscope, and the images were captured with a 40x magnitude. The number of live cells was measured by an automatic cell counter device after three days of music treatment with three different ranges of music frequency. In the automatic cell counter device, live cells are stained in green, and dead cells are stained in red.

The results in Figure 3 demonstrated that music with low frequency increased the number of live cancer cells, while high-frequency music decreased the number of cancer cells. By comparing the live cell number from the control group (No music) and group 1 (river flows in you), the number is significantly higher in group 1 than in the control group. While the control group had 4.3 x 10^4 live cells, group 1 had approximately 5.0 x 10^4 cells, highlighting the effect of low-decibel music that increased the number of live cancer cells. On the other hand, the number of live cells in group 3 (This is what it feels like) was approximately 4.1 x 10^4 cells, which is significantly lower than the number of cells from the control group (4.3 x 10^4 cells). The P-value, which measures how likely any observed difference between groups is due to random chance, was 0.0002 between the control group and group 1 and 0.0045 between the control group and group 3. With a P-value lower than 0.05, it can be concluded that low-decibel music may enhance the cell proliferation of brain cancer cells, while higher decibel may induce cell death of brain cancer cells.

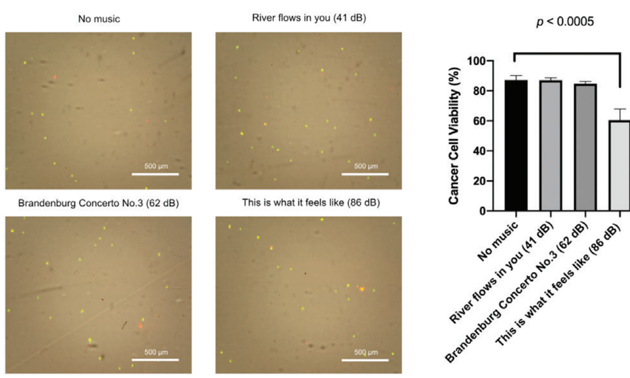

Figure 4: High-decibel music (This is what it feels like) significantly decreased the cancer cell viability after three days of music treatment. This result indicates that high-decibel music induces a significant level of cell death in brain cancer cells.

Figure 4 aims to quantify the viability of brain cancer cells, measured in percentages after the cells are treated with the three different music. As explained in Figure 2, cancer cell viability was measured using the automatic cell counter device, which showed green stains for live cells and red for dead cells. The percentage of cancer cell viability indicates the number of healthy, live cells in the sample and can be calculated using the formula:

$$\frac{Number\ of\ Live\ cells}{Total\ number\ of\ cells\ (live + dead\ cells)} * 100$$

This formula calculates the percentage of live cells relative to the total number of cells in the sample. A viability percentage above 80% indicates a healthy culture, while less than 80% may indicate cytotoxicity.

The results in Figure 4 demonstrated that music with low and middle frequencies did not significantly impact cell viability. In contrast, music with the highest frequency induced a significant level of cell death. The cell viability percentage for the control group was 84.0%, and the viability for group 1 and group 2 were 85.7% and 84.6%, respectively. In other words, more than 80% of the cancer cells in these samples were alive after the music treatment, implying that the music treatment did not significantly impact cell death. The significantly higher number of live cells in group 1 compared to the other groups represented in Figure 2 indicates the speed-up in the cell cycle, which led to a more rapid cell division and higher cell viability.

On the other hand, group 3, which had the highest decibel, had a cell viability percentage of less than 70%, which is significantly lower compared to groups 1 and 2. The viability percentage of 69.0% suggests that the music treatment significantly provoked cell death and killed more than 30% of the existing cancer cells. With a P-value lower than 0.0005, it can be concluded that high-decibel music induces a significant level of cell death of brain cancer cells.

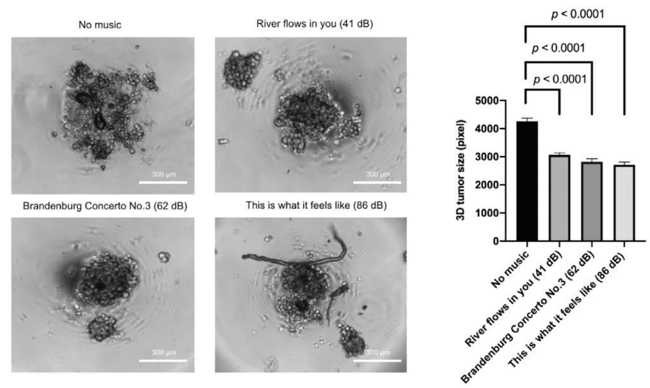

Figure 5: The music treatment resulted in a significant decrease in the size of the primary 3D tumor compared to the group that did not receive music therapy. This result indicates that all music treatment frequencies effectively detached the cancer cells into smaller masses in the 3D culture condition.

Figure 5 measures each cell's 3D tumor size, measured in pixel units, after being treated with three different types of music. Unlike 2D cell structures, which only exist in 2 dimensions, the growth of 3D cell cultures allows for a better representation of cells inside human tissue. The difference between 2D and 3D cell cultures is significant in biological research, particularly in studies investigating the effects of treatments on cancer cells. In a 2D culture, cells are grown on a flat surface, allowing them to spread only in two dimensions. This type of culture is limited in its ability to accurately represent the complex environment found within human tissues, where cells interact in three dimensions. As a result, 2D cultures often fail to mimic the actual behavior of cells in the body, particularly in how they grow, respond to treatments, and interact with their surroundings. On the other hand, 3D cell cultures, such as those described in Figure 5, provide a more realistic model by allowing cells to grow and interact in all three dimensions. This closer approximation to the natural environment of cells within the human body leads to more accurate observations, particularly in cancer research.

The figure consists of 3D tumor images for the four groups (control, groups 1, 2, and 3), which were captured using a mi-

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croscope with a 40x magnitude. The hanging drop technique, a 3D culture method that allows the drops of medium to be maintained with minimal evaporation and without spreading, was performed and maintained for 24 hours to provide enough time for creating the 3D structure of the tumor. Once 24 hours had passed, each group was treated with music (or incubated without any interruption of music for the control group) for another 24 hours. After the treatment, the tumor size was determined by measuring the largest piece of the tumor in pixel size.

The results in Figure 5 highlighted that all three groups treated with music, no matter the length of the sound waves, resulted in a broken 3D tumor structure with several clumps of cells separated from each other. Therefore, all three groups showed smaller tumor sizes than the control sample, which had a relatively stable 3D structure with no cell separation. The tumor size for the control group was more than 4,000 pixels. However, none of the three groups had a tumor size greater than 3,500 pixels. The data shows that as long as the music was playing, sound waves directly vibrated the 3D tumor, which caused a damaged tumor structure. With a P-value lower than 0.0001, it can be concluded that all music treatment frequencies effectively detached the cancer cells into smaller masses. In other words, physical vibration created by sound waves may effectively treat brain cancer.

Conclusion

In summary, using the data represented in Figures 3, 4, and 5, it can be concluded that there is a significant difference between the results of 2D and 3D structures of the tumor cells. Figures 3 and 4 summarize the data from 2D cell culture, highlighting the impact of high-frequency music on the tumor cells. The data from Figure 3 demonstrated that music with high music frequency decreased the number of live cancer cells, and this claim can be supported using the data from Figure 4 as it shows that high music frequency induced significant levels of cell death; there was a significantly lower cell viability percentage for group 3 (high frequency) compared to group 1 and 2 (low and middle frequency). The number of live cells and cell viability percentage for group 3 was also significantly lower than that of the control group. In contrast, groups 1 and 2 did not significantly impact cells.

On the other hand, Figure 5 summarizes the data from 3D cell culture, providing different data compared to Figures 3 and 4. The 3D structure of tumors was unstable and broken into multiple clumps of cells for groups 1, 2, and 3, while the control group maintained a relatively stable and robust structure. This shows that soundwaves may affect the 2D and 3D cells differently, as 2D culture is performed on an artificially made flat surface, while 3D culture is performed in an environment closer to the human tissues. Therefore, the sound waves vibrate the whole 2D structure, while only the outer layer is impacted for 3D cells. Considering that 3D structures share similar properties to human body cells, it can be concluded that physical vibration by sound waves may be an effective treatment for brain tumors.

The biological mechanism by which high-frequency and high-decibel music induces cancer cell death likely involves the mechanical disruption of cellular structures and the triggering of stress responses within the cells. High-frequency sound waves can cause physical vibrations at the cellular level, potentially leading to membrane rupture, cytoskeletal damage, or the disruption of intracellular organelles. These physical disturbances can initiate a cascade of stress responses, including activating apoptotic pathways, where cells undergo programmed cell death.

Some acknowledged limitations in this study need further study. First, only one neuroblastoma cancer cell was used in this study. This may cause the results to be specific to the neuroblastoma cells rather than any other brain cancer cells. Multiple types of brain cancer cells should be tested to validate our results further. Also, the mouse model should be tested in the future to validate the experimental result. Secondly, as mentioned earlier, there are different results in cells from 2D and 3D cultures. Since actual human tissue cells are structured similarly to those from 3D culture, more 3D culture experiments are required to confirm these results. Finally, all the cells were kept in the same incubator a few distances away. This may not have wholly separated the cells from each other, leading to one music affecting different cells. Therefore, the cells should be kept in separate incubators in the future for a completely isolated environment, thus validating our results.

We analyzed the effect of music treatment on brain cancer neuroblastoma cells with a range of low to high music frequencies. Both 2D and 3D data showed that higher music frequency inhibits cell growth relative to lower music frequency. Higher music frequency releases shorter yet stronger sound waves, which induce physical vibration and thus lead to cancer cell death. Overall, this research can be applied in treating brain cancer by breaking the structure of brain tumors with high-frequency music.

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Jonathan Park is a junior at Hong Kong International School. He is an active member and leader of the high school strings orchestra. He hopes to extend his passion for biomedical science by exploring the intersectionality between music and biology.

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