The effect of graphydine oxide (GDYO) nanosheets on disrupting the F-actin filament of anaplastic thyroid carcinoma (ATC) cells

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Abstract

Anaplastic thyroid carcinoma is deadly cancer with high mortality rates. Previous studies have shown that MART-10, an analog to vitamin D, effectively prevents cancer's metastatic potential by downregulating N-cadherin and filamentous actin filaments (F-action filament). In more recent studies around AML (acute myeloid leukemia), a novel material name graphene oxide nanosheets (GDYO) are effective against AML related to DNA(cytosine-5)methyltransferase 3A(DNMT3A)-mutated genes. It has also been found that GDYO nanosheets have been able to disrupt the F-actin filament of AML cells, inducing cell apoptosis which leads to the destruction of the mutated cells. This paper explores the further application of this material in the biomedical field by testing the ability of GDYO nanosheets to induce cell apoptosis in other types of cancer, for example, ATC cells. The study will use an 8505C cell line with negative phosphate-buffered saline (PBS) and positive control Nexavar, a clinically tested drug that treats early ATC cell growth. Nexavar, also known as Sorafenib, inhibits tumor growth by a dual mechanism, acting directly on the tumor (through inhibition of Raf and Kit signaling) and tumor angiogenesis (through inhibition of VEGFR and PDGFR signaling). The paper investigates whether GDYO can successfully disrupt the F-actin filaments of ATC cells, thereby killing them through inducing apoptosis and preventing the metastatic potential of ATC cells.

Keywords: ATC, cell apoptosis, GDYO nanosheets, F-actin filaments

1. Introduction

Anaplastic thyroid cancer is a highly aggressive malignant tumor, also known as anaplastic thyroid carcinoma or undifferentiated thyroid carcinoma. Although only found in 2 to 3 percent of all thyroid cancer neoplasms, it accounts for more than half of the deaths from thyroid cancer in the USA [1]. The overall survival statistics of this specific cancer is around six months on average and only a 20% survival rate over 12 months [2]. Symptoms of this deadly disease include coughing blood out of your mouth, trouble swallowing, lumps on the lower section of your neck, and more [2]. Due to its fast-growing and unpredictable nature, the current best solution is removing cancer from your body through surgery. Although chemotherapy and radiotherapy can still be applied after metastasis, both methods have been shown to give little help to the aggravation of cancer.

In research conducted in 2015, a group of researchers found that MART-10, a new class of less calcemic vitamin D analogs, has shown great possibilities in its ability to inhibit the metastatic potential of anaplastic thyroid cancer. To summarize the article, MART-10 successfully stopped the migration and invasion of ATC cells by reversing the cadherin switch (upregulation of E-cadherin and downregulation of N-cadherin), which led to the attenuation of the EMT process and decrease of F-actin formation [3]. Cadherins are transmembrane proteins that mediate cell-cell adhesion in animals [4]. Cadherins play a crucial role in tissue morphogenesis and homeostasis by regulating contact formation and stability [4,5]. However, during carcinogenesis, cadherins have often been found to be inactive or blocked, allowing cancer to progress into its metastatic stages. As shown in the researcher's experiment, at 10^{-7} molars of concentration, MART-10 can repress 8305C ATC cells' migration by 40±3.1% [3]. However, this article only stated how MART-10 could suppress the migration of ATC cells. The report didn't provide information on whether MART-10 can be applied directly to eliminate cancerous cells.

In 2022, there was a study around GDYO nanosheets on acute myeloid leukemia (AML), a type of bone marrow cancer. In AML, the myeloid stem cells usually become a type of immature white blood cell called myeloblasts (or myeloid blasts). The myeloblasts in AML are abnormal and do not become healthy white blood cells. Sometimes in AML, too many stem cells become abnormal red blood cells or platelets. These abnormal white blood cells, red blood cells, or platelets are also called leukemia cells or blasts. Leukemia cells can build up in the bone marrow and blood, so there is less room for healthy white blood cells, red blood cells, and platelets. When this happens, infection, anemia, or easy bleeding may occur. The leukemia cells can spread from the blood to other body

parts, including the central nervous system (brain and spinal cord), skin, and gums [6]. GDYO nanosheet is a novel, two-dimensional carbon allotrope of an extensively conjugated system [7]. A conjugated system is a system of connected p-orbitals with delocalized electrons in compounds with alternating single and multiple bonds, which in general, may lower the overall energy of the molecule and increase stability [7]. This conjugated system is vital in drug delivery and treating cancer because this allows GDYO nanosheets to bind to various ligands and act as a ligand to bind onto overexpressed or mutated receptors in cancer cells. In this study, the researchers have found that GDYO nanosheets have been shown to kill DNMT3A-mutant AML cells selectively. DNMT3A is one of several epigenetic modifiers identified as recurrently mutated in AML and provides instructions for making an enzyme called DNA methyltransferase three alpha. This enzyme is involved in DNA methylation, which is the addition of methyl groups, consisting of one carbon atom and three hydrogen atoms, to DNA molecules. In clinical studies, DNMT3A mutations have been associated with shorter overall and recurrence-free survival in AML. Thus, DNMT3A represents one of the most critical tumor suppressor genes in hematological malignancies within leukemia patients [8]. Mechanistically, it is discovered that GDYO nanosheets interact with adhesion molecules, ITGB2 and MRC2, which facilitates cellular uptake of GDYO [8].

Moreover, mRNA levels of actin cytoskeleton-associated genes were markedly reduced after GDYO treatment, including ROCK (Rho-associated coiled-coil containing protein kinase), a serine/threonine kinase family that regulates the formation of actin stress fibers38, and PI3K catalytic subunit p110α, which affects filamentous actin (F-actin) polymerization39. The further western blotting analysis confirmed that GDYO diminished the protein levels of ROCK1, ROCK2, and p110α in a dose-dependent manner. This meant that GDYO nanosheets bind to actin and disrupt the actin filaments' organization, eventually leading to differentiation and apoptosis in AML cells [8].

Since GDYO profoundly inhibited actin polymerization, 1μg of GDYO reduced the percentage of polymerized actin as low as latrunculin An [8]. Latrunculin A is a toxin and one of the most widely used reagents to depolymerize actin filaments in live cells [9]. Increased amounts of GDYO depolymerized F-actin comparable to cytochalasin D. This compound binds F-actin at the barbed end to induce actin depolymerization, suggesting GDYO readily interacted with actin monomers and prevented G-actin and F-actin polymerization [8].

It remains a question whether GDYO nanosheets can also induce cell apoptosis in anaplastic thyroid carcinoma by disrupting the actin filament formation. With more research into GDYO nanosheets and closer reading into the details of both papers, it can be predicted that GDYO nanosheets could be a plausible solution to eliminate ATC cells. In both articles, they both mentioned the F-actin filament, a vital component of our plasma membrane. The F-actin filament is essential for living organisms as it is responsible for jobs such as providing mechanical support, determining cell shape, and allowing movement of the cell surface, thereby enabling cells to migrate, engulf particles, and divide [10]. Cells with compromised F-actin filaments will not survive as they cannot maintain their shape and size. This means that the cell will go through cell apoptosis, a process in which the cell goes through a highly intricate and regulated process of cell death, where enzymes called caspases which degrade proteins, are activated [11]. Caspases inside the cell will start catalyzing the enclosure and eventually killing it [12]. Since cell apoptosis won't be stopped once initiated, only specific signals can activate it within an organism.

Since graphdiyne oxide (GDYO) nanosheets have been shown to disrupt filamentous actin(F-actin) filament in the cytoskeleton of acute myeloid leukemia (AML) cells, I predict that adding increasing amounts for various durations with GDYO nanosheets results in cell killing of 8505C sensitive thyroid cancer (ATC) cells. I will measure the size of the ATC tumor from 8505C xenograft with nanosheet treatment and measure Annexin V/PI of nanosheet-treated 8505C cells in culture actin filament changes by immunofluorescence of 8505C cells treated with nanosheets in vitro. My negative control is phosphate-buffered saline (PBS). My positive control is Nexavar, a clinically tested drug that treats ATC cells.

2. Material

2.1 *Cell culture*

Human thyroid carcinoma cell lines, 8505C, were purchased from ATCC. 8505C cells were grown in RPMI-1640 supplemented with 10% fetal bovine serum (FBS). The culture medium was changed five times per week. The cell will be kept at 8% CO_2 concentration and 90% O_2 concentration. The temperature will be held around 37°C. Harvested cells were cultured in StemSpanTM SFEM (Stemcell Tec.) supplemented with SCF (50 ng/mL) and TPO (50 ng/mL) [13].

2.2. *Synthesis of GDYO*

GDYO was prepared by acid-mediated oxidation of GDY by a modified Hummers method. In brief, 10 mg GDY power was added to the solution of concentrated 1 mL

HNO3 and 3 mL H2SO4 in a three-necked flask, and then 10 mg KMnO4 was slowly added to the mixture under vigorous stirring in an ice bath. After cooling to room temperature, the mixture was transferred to an oil bath at 80 °C and vigorously stirred for 24h to give a brown suspension. After being cooled to room temperature, the rest was ultrasonicated for 10 min, and the pH was adjusted to 8.0 with NaOH. Then, the product was collected by centrifugation at 10,000 g for 10 min and washed with pure water three times. The resulting product was re-dispersed into ultrapure water and ultrasonicated for about 12 h to obtain a homogeneous brown aqueous dispersion [8].

2.3. *Mice*

C57BL/6J and NSG mice were purchased from Jackson Laboratory. Male and female mice from 8 to 12 weeks old were used for all studies. All mice were housed under specific pathogen-free conditions. All mice were cultured in suitable temperature and humidity environments and fed sufficient water and food (25 °C, appropriate humidity (typically 50%), dark/light cycle for 12 h). All animal experiments were performed according to protocols approved by the Norwegian National Research Ethics committee [8].

2.4. *pH Titration*

As previously reported, the concentration of ionized groups on GDYO and GO sheets at different pH values was determined using a pH titration. 0.1 g of GDYO/GO was taken in a beaker containing 20 mL of 0.1 M NaOH solution and 0.1 M HCl solution added in incremental steps of 0.25 mL. At each stage, the pH of the solution was recorded after ensuring that equilibrium had been attained. The experiment was repeated with the same volume of NaOH but now without additional GDYO/GO. The difference in the books of HCl in the titration curves for the same pH value gave the concentration of the ionized groups per g of GDYO/GO at that pH [8].

2.5. *Flow cytometry analysis*

Apoptosis was detected by Annexin V and PI staining. Cells were pre-treated with GDYO or GO (20 μg/mL-50 μg/mL) for a time between 48-72 h and washed twice by pre-cooling PBS. 1×10^5 cells were collected and resuspended by the binding buffer, Annexin V and PI were added into cell suspension. After 20 min incubation, flow cytometry was detected in the FITC and PE channels. For ATC stem cells, cells were stained with antibodies against CD133, LNK, KRT8, and PRAR with a human lineage cocktail (CD3, CD14, CD19, CD20, CD56). Antibody staining was performed at four °C for 45 min[8]. The experiment is repeated three times.

2.6. *Mouse xenograft*

1×10^6 live cells were transplanted into NSG mice via tail vein injection. For the negatively controlled experiment, PBS was injected into the rat for 48 h-72h. The same procedure will be repeated with the positive control Nexavar. For GDYO treatment, GDYO was injected via the tail vein every two days, starting at a dose of 0.2 mg per mouse. The increment between the two days will be 0.1 mg more than the last test. ATC cell development was monitored by in vivo bioluminescence imaging with a small-animal imaging system (IVIS Spectrum, PerkinElmer). The mice were injected with D-luciferin firefly (PerkinElmer) at 3 mg per mouse. The bio-luminescence images were acquired 15 min after injection. Mice were sacrificed randomly at day 31 (n = 6), and the thyroid was removed and weighed. The thyroid was then analyzed by flow cytometry. The remaining mice (n = 6) were raised to calculate the survival curve up to 50 days [8].

2.7. *F-actin immunofluorescence staining and live imaging*

ATC cells were attached to fibronectin-coated glass bottom dishes. After GDYO/GO treatment, cells were washed and fixed in 1% paraformaldehyde at room temperature for 15 min. Cells were then permeabilized with 0.2% Triton X-100 at room temperature for 30 min. To visualize F-actin, cells were stained for 30 min with Alexa Fluor 488 phalloidin (Invitrogen). For LAMP1 staining, cells were stained with primary LAMP1 antibody overnight, washed twice, and stained with FITC-labeled secondary antibody. Cells were mounted on glass slides with Fluro-gel mounting media containing DAPI and covered with glass coverslips. Images were taken with a confocal microscope (Nikon A1R) using a 63×1.4NA oil objective. ATC cell lines OCI-AML3 and HL-60 expressing Lifeact-tdTomato were attached to fibronectin-coated glass bottom dishes overnight. GDYO or GO was added at zero time point, and TIRF images were taken over 48 h with an interval of 10 min. Multiple cells (n = 20 for each group) were photographed to quantify, and the fluorescence intensity of ROI was measured by ImageJ 1.48 v [8]. The experiment is repeated three times.

2.8. *Statistical analysis*

Each experiment was repeated at least three times independently. All the data are represented as mean ± standard deviation (SD). Student's t-test was used for statistical analysis of two groups, and one-way ANOVA followed by Tukey's posthoc tests was used for comparisons among multiple groups and differences among various groups. Survival data were determined for

every group by the Kaplan–Meier method and compared by the log-rank (Mantel-Cox) test. The P value was calculated using GraphPad Prism 8. Those <0.05 were considered significant [8].

3. Possible Results

3.1. *Flow cytometry*

1. **The ATC cells treated with GDYO nanosheets did go through cell apoptosis and lost membrane integrity.** An indication of cell apoptosis and membrane integrity loss after being treated with GDYO nanosheet indicates that the first GDYO nanosheet can eliminate ATC cells by inducing cell apoptosis [12]. It can be inferred that the mechanism is similar to how it suppresses AML cells, disrupting the F-actin filament inducing cell apoptosis, and causing cells to lose their membrane integrity [14].

3.2. *The ATC cells treated with GDYO nanosheets didn't go through cell apoptosis ye still lost membrane integrity*

An indication of no cell apoptosis yet a loss in membrane integrity after being treated with GDYO nanosheet indicates that first, GDYO nanosheets can eliminate ATC cells by causing ATC cells to lose their membrane integrity in ways that are not apoptosis [2].

3.3. *The ATC cells treated with GDYO nanosheets didn't go through cell apoptosis and didn't lose membrane integrity*

An indication of no cell apoptosis or loss in membrane integrity after being treated with GDYO nanosheet indicates that GDYO nanosheet could not eliminate ATC cells by any means [14].

3.4. *GDYO Nanosheets has shown to significantly decrease the size of ATC tumors more than the positive control Nexavar*

A decrease in length greater than Nexavar indicates that not only does GDYO work in cell lines, but it also effectively eliminates ATC cells in vivo.

3.5. *GDYO nanosheets has shown to decrease the size of ATC tumors; however less effective than Nexavar*

A decrease in length that is less than Nexavar indicates that not only does GDYO work in cell lines, but it also helps to eliminate ATC cells in vivo. This also means that GDYO nanosheets show some real therapeutic in eliminating ATC cells.

3.6. *GDYO nanosheets fail to decrease the size of the ATC tumor, and the result is the same as the negative control PBS*

The exact size of the ATC cell as the PBS indicates that GDYO nanosheets cannot eliminate ATC cells in vivo.

3.7. *GDYO nanosheets increase the size of ATC tumors instead of decreasing them.*

An increase in the size of the ATC cell indicates that the GDYO nanosheet can stimulate ATC cell growth in vivo.

3.8. *ATC cells F-actin filament show no disruption after GDYO treatment*

F-actin fluorescence, which shows no trouble after GDYO treatment, indicates that the GDYO nanosheet does not trigger cell apoptosis and doesn't affect the F-actin filament in ATC cells [15]. This refutes the hypothesis [16].

3.9. *ATC cells F-actin filament shows minor disruption after GDYO treatment that would not cause apoptosis*

F-actin fluorescence, which offers little trouble, indicates that the GDYO nanosheet does have some effect on the F-actin filament. Still, it is not enough to induce cell apoptosis, eliminating the ATC cells.

3.10. *ATC cells F-actin filament shows significant disruption after GDYO treatment that would lead to cell apoptosis*

F-actin fluorescence, which shows considerable trouble, indicates that GDYO nanosheets have a substantial amount of influence in actin filament in ATC cells, one that is significant enough to induce cell apoptosis also suggests the potential for GDYO nanosheets to eliminate ATC cells [15] (Table 1).

Table 1: Combination Possible Result Chart (CR

Possible results	CR 1	CR 2	CR3	CR4	CR5	CR6	CR7	CR8
Increased Annexin V and PI by FACS?	+	-	+	+	-	-	+	-
Disruption of F-actin filaments increases?	+	+	-	+	-	+	-	-
Mouse xenograft tumor size decreases?	+	+	+	-	+	-	-	-
Support Hypothesis	Yes	Partially	Partially	Partially	Partially	Partially	Partially	No

Note. "+" means a result that is the same or similar to the positive control Sorafenib, and "- " means an impact that is the same or equal to the negative control PBS.

3.11. *Possible result of concentration and duration (PRC/PRD)*

PRC 1: As the concentration of GDYO nanosheets increases, the number of ATC cells killed by GDYO nanosheets correlates with attention.
PRC 2: As the concentration of GDYO nanosheets increases, the number of ATC cells killed by GDYO nanosheets shows no change.
PRC 3: As the concentration of GDYO nanosheets increases, the number of ATC cells killed by GDYO nanosheets has an inverse relationship with the attention.
PRD 1: As the duration of GDYO between each time it was given in mouse xenograft increases, the size of the ATC tumor decreases significantly.
PRD 2: As the duration of GDYO between each time it was given in mouse xenograft increases, the size of the ATC tumor doesn't change significantly.
PRD 3: As the duration of GDYO between each time it was given in mouse xenograft increases, the size of the ATC tumor increases.

4. Discussion on individual experiment results

Although previous studies have confirmed the ability of GDYO nanosheets to bind to DNMT3A mutated cells, research hasn't been conducted around applying GDYO nanosheets to various types of cancer. Since GDYO nanosheets have been shown to bind to and disrupt the F-actin filament of AML cells to induce apoptosis, it is safe to conclude that the state of the actin filament is crucial to the survival of cancer cells. In possible results 1 and 10, we can see a close relationship between the actin filament and the cell membrane. In both results, the cell has entered into apoptosis due to a membrane integrity loss due to a disruption in F-actin filaments. This aligns with my hypothesis that GDYO nanosheets disrupt F-actin filaments and induce cell apoptosis.
In possible result 2, a loss of cell membrane integrity without going through apoptosis indicates that the GDYO nanosheets induce a different cancer cell mechanism in ATC cells. In this case, the type of cell death that ATC cells will enter will probably classify as necrosis. Necrosis is usually considered a passive or accidental spill out of cellular content through environmental perturbation. The GDYO nanosheets may have been able to bind to ATC cells and introduced mechanical stress through its binding with actin filaments, resulting in passive cell death that doesn't require energy to activate specific signal pathways, which is necrosis. This result would partially support my hypothesis as GDYO nanosheets can kill ATC cells but in a different method than how it kills AML cells [15].
As a possible result 3, ATC cells were not affected by GDYO nanosheets as they didn't lose their membrane integrity. This will disprove my hypothesis as this result implies that GDYO nanosheets do not kill ATC cells. Its ability to bind to F-actin filaments is specific to certain proteins and receptors in AML cells but not ATC cells. These results also imply that GDYO nanosheets can't be used as a general treatment for most cancers as they only bind to specific receptors at specific cells in the body.
In possible results 4 and 5, the results indicated that GDYO nanosheets possess clinical significance in eliminating ATC cells. In both results, there is a decrease in the tumor size in rats that get GDYO nanosheets injected compared to the rats injected with the negative control PBS. Although the hypothesis favors both results, in possible result 4, a decrease in size more significant than the positive control indicates that GDYO nanosheets are better at killing ATC cells than current medications. This meant that GDYO had a definite advantage in eliminating ATC cells, suggesting a sizeable production of this cure if passing the biosafety test. However, in possible result 5, a smaller size than the positive control indicates that although GDYO nanosheets can effectively eliminate ATC cells, the current medicine is still more effective. This means that unless GDYO has advantages in other fields, it will not be used a lot since the recent medication work the best.
In possible results 6, A change in the tumor size similar to the negative control indicates that GDYO nanosheets have neither a positive nor a negative impact on the growths of the ATC cells. This result implies that the GDYO nanosheet is not involved in the tumor suppression or cancer cell elimination process, which does not support the hypothesis. In possible result 7, an increase in the tumor size that is bigger than the negative control indicates that GDYO not only doesn't suppress the growth of the tumor, it increases the tumor's growth rate. This result also disproves the hypothesis as the hypothesis predicts that GDYO nanosheet can reduce the number of ATC cells, thereby decreasing the tumor size.
In possible result 8, no disruption of the F-actin filaments indicates that the GDYO nanosheet does not bind to actin filaments in ATC cells. First, this meant that the GDYO nanosheet would not be able to induce cell apoptosis in ATC cells as without attaching to the cytoskeleton where the actin filaments are located, GDYO can't interact with any signaling pathways that trigger cell apoptosis in ATC cells. Secondly, this meant that for GDYO to bind to the F-actin filaments of a cell successfully, specific receptors must bind to GDYO nanosheets in DNMT3A mutated AML cells which are not present in ATC cells.

This does not support the hypothesis as this result denies the possibility for GDYO nanosheets to apply to most cancers. It also shows how GDYO nanosheets do not affect suppressing ATC cell growth or killing GDYO nanosheets.

In possible result 9, a minor disruption on the F-actin filament that is not enough to result in cell apoptosis indicates that GDYO nanosheets can bind to various cancers due to its conjugated system. However, this only partially supports my hypothesis, as although GDYO nanosheets can bind to F-actin filaments, it wasn't able to create a significant amount of impact on the ATC cells to kill ATC cells completely. This could either mean that ATC cells are structurally different from AML, which results in varying levels of disruption on the F-actin filaments to induce cell apoptosis, or it can simply mean that GDYO nanosheet can only induce cell apoptosis in AML through binding in specific receptors on the surface of AML cells.

5. Discussion of combined results

The possible results were then combined to create a general overview of the effect of GDYO nanosheets on ATC cells. Combination 1 is in line with the hypothesis that GDYO binds to and disrupts F-actin filaments, inducing cell apoptosis and thereby decreasing the size of the ATC tumor in vivo.

Combinations 2-7 partially agree with the hypothesis. In combinations 2 and 5, a lack of cell apoptosis yet observed decreased tumor size indicates that GDYO nanosheets effectively kill ATC cells. However, this result implies that the mechanism of cell death that GDYO imposes on ATC cells differs from the mechanism of cell death that GDYO sets on AML cells. Further investigation should be conducted to discover why and how GDYO induces different cell death mechanisms in other cells. In combination 3, a lack of F-actin filaments yet observed cell apoptosis and decrease in ATC tumor size in vivo indicates that instead of directly binding to the ATC cells, which is a way of inducing cell apoptosis, GDYO bind to other types of cell signal pathways which is related to cell growth and cell death. Further research should be done on the exact path that GDYO nanosheet binds in ATC cells and why GDYO differs in how to introduce cell apoptosis at different cells.

In combinations 4, 6and 7, observed cell apoptosis or disruption of F-actin filaments that didn't reduce the size of the ATC tumor in vivo indicates that GDYO nanosheet is binding to non-cancerous cells inside the body. It is killing them instead of the ATC cells it supposes to kill. This result could mean that ATC tumor has a unique mechanism that makes GDYO nanosheets unable to bind to them, causing GDYO nanosheets to flow into the bloodstream and potentially killing other healthy cells. This result could also mean that GDYO nanosheets bind nonspecifically in vivo, binding to any cells. This is very dangerous and lethal. If GDYO nanosheets cannot secure specifically against cancer, they will kill all the cells inside the human body, which is detrimental to our body. In either scenario, further investigation should be conducted to ensure the safety of GDYO nanosheets and how to mimic Sorafenib's effect on ATC cells through GDYO nanosheets.

Combination 8 opposes the hypothesis that was made. Not only was there no cell apoptosis and no F-actin filament disruption, but the size of the ATC tumor also didn't decrease when GDYO nanosheets were injected. This result implies that the GDYO nanosheets do not affect suppressing or eliminating ATC cells. Their ability to induce cell apoptosis is limited to specific cancers due to differences in the mutation in a gene in a different type of cancer.

6. Discussion on possible results of concentration and duration

In the possible result of concentration (PRC) 1, the increase of concentration of GDYO, which leads to a proportional increase in the number of ATC cells, indicates that GDYO is not only related to the elimination of ATC cells but also represents how an increase in GDYO will lead to more ATC cells killed. When GDYO concentration increases, more GDYO nanosheets are ejected into cells and animals. This means more ATC cells will have GDYO nanosheets attaching to the F-actin filament, inducing cell apoptosis and killing ATC cells.

In PRC2, the increase in the concentration of GDYO nanosheets, which resulted in no change in the ATC cells that were killed, indicates that the substance that was killing the ATC cells might not be GDYO nanosheets. Further investigation should be performed around the sense that came into contact with ATC cells to find out what is killing ATC cells.

In PRC3, an increase in the concentration of GDYO nanosheets that leads to a decrease in the number of ATC cells killed indicates that as GDYO increases, its effect on killing ATC cells decreases. A possible explanation is that GDYO nanosheets are also an allosteric regulator and a ligand for ATC cells. As GDYO nanosheets bind to the F-actin filaments of ATC cells, it can change the conformation of the actin filament of the cell it is attached to and also the cells around it, causing later GDYO nanosheets to be unable to bind to ATC cells, thereby

reducing overall ATC cells killed by GDYO nanosheets.

In a possible result of duration (PRD)1, an increase in the duration between each time GDYO nanosheets were given in mouse xenograft leads to a significant decrease in ATC tumor size, indicating that the length of course between each time mouse was given GDYO nanosheet, it kills more ATC cells. A possible explanation for this phenomenon is that it takes an extensive period for GDYO nanosheets to bind to the F-actin filaments of ATC cells, which means that if there is a more prolonged period in between each time, GDYO nanosheets are given, it will improve the overall effect of GDYO nanosheets by reducing the size of ATC tumor.

In PRD2, an increase in the duration between each time GDYO nanosheets were given in mouse xenograft leads to no or insignificant changes in the tumor size, indicating that time is not a variable that determines the amount of ATC cells killed by GDYO nanosheets. This result means that no matter how long in between GDYO cells was given, the decrease in the size of the tumor is always similar.

In PRD3, an increase in the duration between each time GDYO nanosheets were given in mouse xenograft leads to an increase in the size of the ATC tumor, indicating that GDYO nanosheets should be taken regularly. A possible explanation is that GDYO nanosheets have minimal time to attach to the ATC cells. This means that if not taken regularly, the effect of GDYO in killing ATC cells can very quickly disappear. This allows freedom for the ATC tumor to grow and leads to the result of the size of the tumor increasing.

7. Conclusion

In conclusion, this research paper explores the possibilities of inducing cell apoptosis in different cancers by binding to and disrupting the F-actin filaments of the cancer cell. The result of this study will indicate whether the GDYO nanosheet can induce ATC cell apoptosis, disrupt F-actin filaments in ATC cells and decrease the size of ATC tumors in vivo. This result would also give insight into the possibility of GDYO nanosheets' capability to kill a massive spectrum of known cancer through its conjugated system. The observed effect in killing ATC cells will also provide necessary knowledge about the different mechanisms that cancer uses to survive in our body and help develop innovative methods and medications to bypass the defensive mechanisms of cancer. Since GDYO nanosheets are just one of the newly invented materials which have been shown to have the ability to bind and kill cancer cells, the result of this research could also lead to the future discovery of more materials that can kill or suppress cancer cells.

Reference

[1] American Thyroid Association. (n.d.). Anaplastic Thyroid Cancer. American Thyroid Association. Retrieved November 3, 2022, from https://www.thyroid.org/anaplastic-thyroid-cancer/

[2] What Is Anaplastic Thyroid Cancer? (2020, May 29). WebMD. https://www.webmd.com/cancer/anaplastic-thyroid-cancer-overview

[3] Chiang, K.-C., Kuo, S.-F., Chen, C.-H., Ng, S., Lin, S.-F., Yeh, C.-N., Chen, L.-W., Takano, M., Chen, T. C., Juang, H.-H., Kittaka, A., Lin, J.-D., & Pang, J.-H. S. (2015). MART-10, the vitamin D analog, is a potent drug to inhibit anaplastic thyroid cancer cell metastatic potential. *Cancer Letters*, *369*(1), 76–85. https://doi.org/10.1016/j.canlet.2015.07.024

[4] Moh, M. C., & Shen, S. (2009). The roles of cell adhesion molecules in tumor suppression and cell migration. *Cell Adhesion & Migration*, *3*(4), 334–336. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2802741/

[5] Kaszak, I., Witkowska-Piłaszewicz, O., Niewiadomska, Z., Dworecka-Kaszak, B., Ngosa Toka, F., & Jurka, P. (2020). Role of Cadherins in Cancer—A Review. *International Journal of Molecular Sciences*, *21*(20), 7624. https://doi.org/10.3390/ijms21207624

[6] National Cancer Institute. (2021, October 1). *Acute Myeloid Leukemia Treatment (PDQ®)–Patient Version - National Cancer Institute*. Www.cancer.gov. https://www.cancer.gov/types/leukemia/patient/adult-aml-treatment-pdq#:~:text=Acute%20myeloid%20leukemia%20(AML)%20is%20a%20cancer%20of%20the%20blood

[7] Guo, X., Huang, H., Cui, R., Wang, D., Liu, J., Wang, D., Liu, S., Zhao, Y., Dong, J., & Sun, B. (2022). Graphdiyne Oxide Quantum Dots: The Enhancement of Peroxidase-like Activity and Their Applications in Sensing H_2O_2 and Cysteine. *ACS Applied Bio Materials*, *5*(7), 3418–3427. https://doi.org/10.1021/acsabm.2c00361

[8] Wang, Q., Liu, Y., Wang, H., Jiang, P., Qian, W., You, M., Han, Y., Zeng, X., Li, J., Lu, H., Jiang, L., Zhu, M., Li, S., Huang, K., Tang, M., Wang, X., Yan, L., Xiong, Z., Shi, X., & Bai, G. (2022). Graphdiyne oxide nanosheets display selective anti-leukemia efficacy against DNMT3A-mutant AML cells. *Nature Communications*, *13*(1), 5657. https://doi.org/10.1038/s41467-022-33410-w

[9] Fujiwara, I., Zweifel, M. E., Courtemanche, N., & Pollard, T. D. (2018). Latrunculin A Accelerates Actin Filament Depolymerization in Addition to Sequestering Actin Monomers. *Current Biology*, *28*(19), 3183-3192.e2. https://doi.org/10.1016/j.cub.2018.07.082

[10] Dominguez, R., & Holmes, K. C. (2011). Actin Structure and Function. *Annual Review of Biophysics*, *40*(1), 169–186. https://doi.org/10.1146/annurev-biophys-042910-155359

[11] Okegawa, T., Pong, R.-C., Li, Y., & Hsieh, J.-T. (2004).

The role of cell adhesion molecule in cancer progression and its application in cancer therapy. *Acta Biochimica Polonica*, *51*(2), 445–457. https://pubmed.ncbi.nlm.nih.gov/15218541/#:~:text=Multiple%20and%20diverse%20cell%20adhesion

[12] Cooper, G. M. (2000). Structure and Organization of Actin Filaments. *The Cell: A Molecular Approach. 2nd Edition*. https://www.ncbi.nlm.nih.gov/books/NBK9908/#:~:text=Actin%20filaments%20are%20particularly%20abundant

[13] Bioprobes 64. (2011, April). *Dead Cell Identification in Flow Cytometry: Eliminate Dead Cells for Increased Accuracy - US*. Www.thermofisher.com. https://www.thermofisher.com/ca/en/home/references/newsletters-and-journals/bioprobes-journal-of-cell-biology-applications/bioprobes-issues-2011/bioprobes-64-april-2011/eliminate-dead-cells-for-increased-accuracy-april-2011.html#:~:text=Loss%20of%20membrane%20integrity%20is

[14] Robert, K. (2021, February 1). *How to Use Flow Cytometry to Measure Apoptosis: Part One*. Bio-Rad. https://www.bio-rad-antibodies.com/blog/flow-cytometry-apoptosis-part-one.html

[15] Green, D. R., & Llambi, F. (2015). Cell Death Signaling. *Cold Spring Harbor Perspectives in Biology*, *7*(12), a006080. https://doi.org/10.1101/cshperspect.a006080

[16] Labatory, B.-R. (2015). *Description*. https://www.bio-rad.com/webroot/web/pdf/lsr/literature/10048733.pdf