The role of PIWI proteins in glioma formation

Ava Serrano

Section 1: Introduction

Glioblastoma (GBM) is a deadly brain cancer with no known cure. Around 10,000 people in the United States are diagnosed with GBM every year, 95% of which die within the first 5 years after diagnosis[1]. Not much is known about the mechanisms of GBM tumorigenesis, however current research shows over 90% of GBM cells have an extreme overexpression of piwi proteins[1]. Piwi proteins typically regulate self renewal pathways in stem and germ cells, but their overexpression disrupts cancer suppressing genes and healthy cell functions.

PIWI-like protein 1 (Piwil1) belongs to the PIWI family of proteins, which are key components of the piRNA pathway. While they are typically expressed in germline development, emerging evidence suggests that these proteins are also aberrantly expressed in many gliomas. The expression of Piwil1 has been observed in both low-grade and high-grade gliomas. Several studies have reported elevated Piwil1 expression in glioma tissues compared to normal brain tissues, suggesting its potential involvement in the development of gliomas [2,3]. Studies have demonstrated that Piwil1 promotes glioma cell proliferation, invasion, and migration, implicating its involvement in tumor growth and metastasis[2]. Piwil1 has been implicated in the dysregulation of tumor suppressing genes and promotion of tumor enhancing genes. Specifically, Piwil1 directly regulates BTG2, FBXW7, and P27, all genes that suppress tumor growth[2]. Piwil1 downregulates these genes resulting in dysregulation of the cell cycle, inhibition of cell growth, and aberrant timing and cell cycle progression. When FBXW7 is mutated or downregulated, MYC is accumulated which drives tumor growth[2]. Other genes are overexpressed due to Piwil1 dysregulation such as CCND2, NESTIN, OLIG2, and MCL1[2]. Elevated levels of these genes and proteins lead to tumor growth and help cancer cells survive. By inhibiting and overexpressing certain genes, Piwil1 helps glioma cancer cells self-renew and glioblastomas grow[2].

This gene dysregulation allows glioma cells to multiply quickly and uncontrollably, contributing to the challenges of GBM treatment. The field linking overexpression of piwi proteins and GBM is still young, thus the functional mechanism between piwi proteins and tumor formation remains unknown. For example, it is unknown whether the signaling from the tumor increases piwi protein expression or whether piwi becomes overexpressed and then induces GBM tumorigenesis. Based on the research I have done using both data-driven and review papers, I hypothesize that piwi overexpression causes the formation of a GBM. This overexpression aids in tumor malignancy thus supporting piwi proteins as oncogenes. This review paper hopes to increase the importance of the relationship between GBM and piwi protein overexpression to promote research to elucidate the functional mechanism between the two. Understanding Piwi's role in glioblastoma could lead to new therapies targeting these proteins and improving patient outcomes[2].

Section 2: Definitions

Glioblastoma: A highly aggressive type of brain tumor originating from glial cells in the central nervous system.

Glioma stem cells: Specialized cells within gliomas that possess the ability to self-renew or differentiate into other cell types.

Piwi: A protein belonging to the PIWI family, involved in the piRNA pathway.

Cancerous cells: Cells that grow and divide uncontrollably forming a mass of cells that can invade nearby tissues and potentially spread to other parts of the body.

Glial cell: Non-neuronal brain cells that provide support and protection.

Metastasis: The spread of cancer cells from the primary tumor to other parts of the body through the bloodstream or lymphatic system, forming a new mass in a new site.

Senescence: Cells at rest (preventing replication).

Low-grade glioma: Slower growing and less aggressive brain tumor.

High-grade glioma: Fast-growing, aggressive brain tumor with potential to spread.

CCND2: Can cause cells to grow uncontrollably, leading to increased cell division, which might play a role in tumor growth.

MCL1: This gene supports the survival of cancer cells. Blocking this gene can aid in stopping tumors

OLIG2: Changes in this gene are connected to specific glioma types and it is more active in gliomas.

NESTIN: This gene is frequently expressed in tumor cells and plays a role in promoting tumor growth, making the tumor cells harder to treat, increasing the chances of the tumor coming back.

BTG2: This gene stops tumor growth and development by limiting cell growth, encouraging cells to mature, and influencing apoptosis.

p53: Alterations or mutations in the p53 gene can disrupt its tumor-suppressing function, which can contribute to the development and progression of brain tumors.

Fbxw7: This gene acts as a tumor suppressor by keeping important control proteins at the right levels. When it is altered or not working properly, it is linked to different cancers.

Myc: Controls many cell activities, like growth and the cell cycle. When it is overactive, it can cause cells to over multiply, leading to cellular instability and bigger tumors.

The following research papers were chosen based on their relevance to dysregulated gene and protein expression in gliomas (Fig 1). PIWIL1 overexpression and its downstream effects resulting in dysregulated genes is the topic of the first paper[2]. The second paper is on one of these dysregulated genes, MCL-1, and how overexpression of this gene contributes to malignant gliomas[3]. Both of these papers used humans and mice in their research in order to understand the relationship between piwi overexpression and glioma tumorigenesis [2,3]. I also looked at a review paper that focused on piRNAs and Piwi expression in cancers. This paper helped me better understand the role of piRNAs in cancer and the relationship between Piwi proteins and piRNAs.

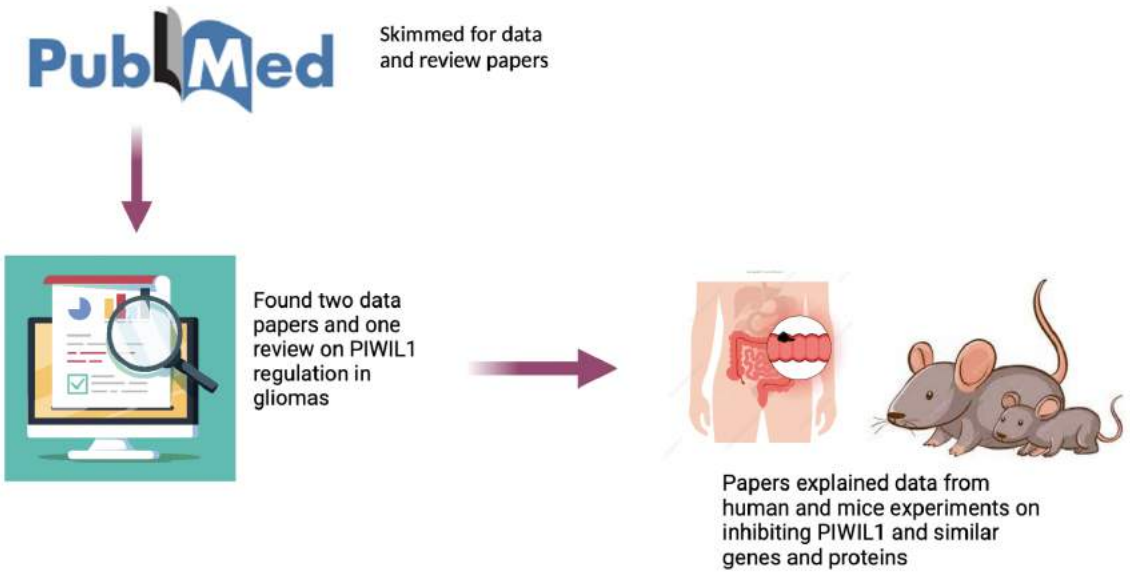

Figure 1. Flow chart of literature review for this review paper.
I did a search on Pubmed for articles relating to Piwi dysregulation in gliomas. This search led me to two informative research papers. The first paper researched the overexpression of PIWIL1 and its significance in gliomas. The second paper focuses on the overexpression of MCL-1 and how it is relevant in the context of gliomas. Both studies employed a combination of mice models and human cell lines, ensuring a comprehensive analysis of PIWIL1 in gliomagenesis.

Section 3: Evidence/Data

One of the first and only papers to identify the relationship of PIWIL1 and gliomas is “Piwil1 Regulates Glioma Stem Cell Maintenance and Glioblastoma Progression”. This paper elucidates genes that were dysregulated by altered PIWIL1 expression and shows a significant correlation between PIWIL1 overexpression and gliomas. However, the authors are still unable to conclude whether there is a connection between PIWIL1 overexpression and glioma tumorigenesis. This study evaluated PIWIL1 levels in both mouse and human subjects. Additionally, glial stem cells derived from human GBM tumors were used to evaluate PIWIL1 roles in GBM. They used two cell lines from human GBM patients in their experiments. In order to attain these cell lines, tumors were isolated from each patient and subsequently cells were harvested and cultured from the tumors. For their model the authors knocked down PIWIL1 in the glioma stem cells (GSC) by using short hairpin RNAs (shRNA) to decrease the amount of PIWIL1 in their cell lines. After knockdown the authors showed that there is an increase in cell viability and decrease in tumor size (fig 2). This knockdown of PIWIL1 demonstrated the importance of PIWIL1 to tumor survival. Since they were able to show that a knockdown of PIWIL1 results in lessened tumor malignancy in cell lines, the authors decided to repeat the experiment in a mouse model using GSC-derived xenografts. They did this by implanting GSCs into the right frontal lobe of mice. To see if the same results still occurred in this model, they also knocked down PIWIL1 using shRNAs. They found that in these knockout xenograft mouse

models, mice with PIWIL1 knockdowns survived longer than their sham shRNA xenograft counterparts. In the knockdown model, they found that there is an increase in cleaved caspase 3 and a decrease in MCL-1. Cleaved caspase 3 helps suppress tumor growth and kill cancer cells, therefore a PIWIL1 knockdown could have therapeutic effects. ShPiwil1 mice also had increased levels of FBXW7 and BTG2, which suppress tumors. This is supported from the data as the mice treated with a sham shRNA died almost immediately and the shPiwil1 mice lived longer and had a lower death rate (Fig 3). Authors found that with a knockdown of PIWIL1, MCL-1 is decreased (fig 3B). This result is promising as MCL-1 promotes tumor growth and cancer viability, therefore a decrease in expression is favorable for a healthy outcome. When there is no PIWIL1 being expressed, the tumor size reduces significantly, leaving almost no tumor as opposed to when there is PIWIL1 and there is a large tumor[2].

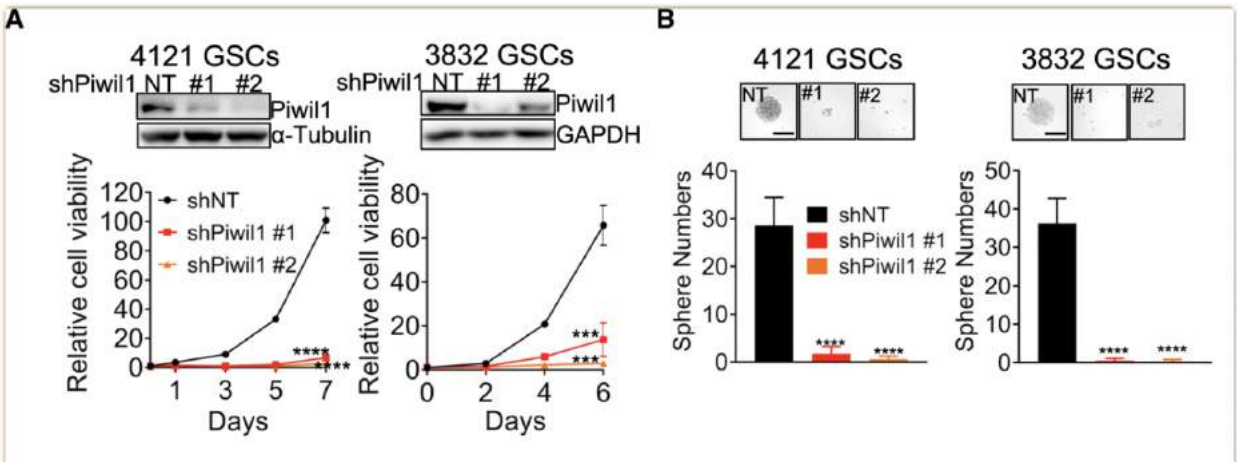

Figure 2. PIWIL1 knockdown in GSC's increases cell viability and decreases tumor size. **A.** Western blot of Piwil1 in GSCs with no shRNA (NT) and PIWIL1 shRNA knock out (#1, #2). Results show a marked decrease of Piwil1 protein in shRNA treated cells. Graph below shows relative cell viability with cells treated with no shRNA, shRNA 1, shRNA2. There is a significant increase in cell viability in cells treated with shRNA compared to shNT. **B.** Representative images of tumor size when PIWIL1 is knocked down compared to when there is PIWIL1 being expressed. There is a significant decrease in tumor size in shRNA cells compared to shNT. Graph below shows quantitative data from the representative images above. Figure adapted from Huang et al. 2021.

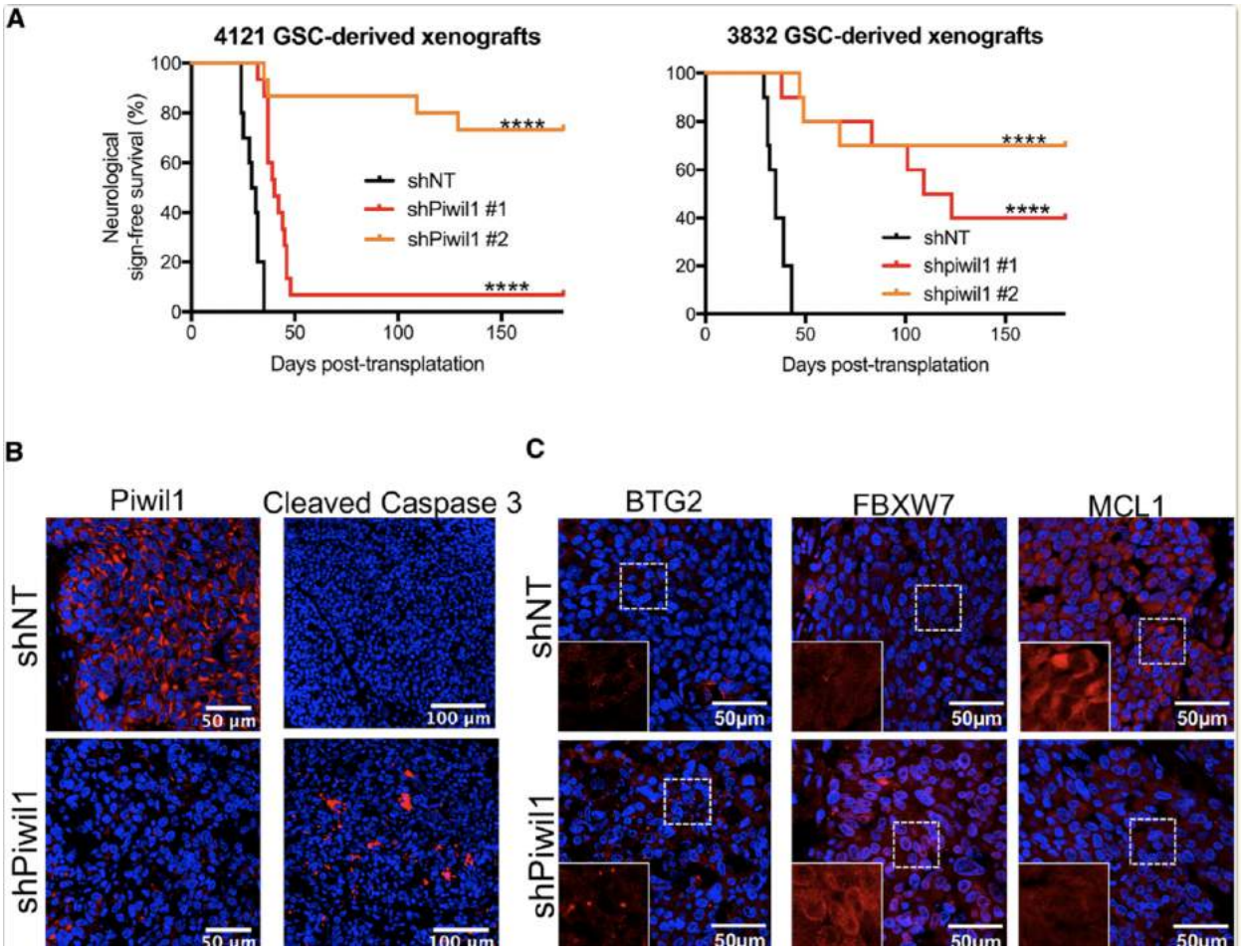

Figure 3. Piwil1 knockdown in mice results in increased mouse survival and aberrant protein expression.
A. Knockdown of PIWIL1 in two GSC lines transplanted in mice; 4121 GSC-derived xenografts and 3832 GSC-derived xenografts. Both shPiwil1 #1 and #2 showed a significant increase in mouse survival for both xenografts. **B.** Immunofluorescence staining against Piwil1 and cleaved caspase 3 in the shNt and shPiwil1 mice. Nuclei are stained with DAPI. Results show a decrease in Piwil1 and an increase in cleaved caspase 3 in shPiwil1 tumors compared to sham knockout tumors (shNT). **C.** Immunofluorescence staining against BTG2, FBXW7, and MCL1 in shNT and shPiwil1 mice. Nuclei are stained with DAPI. Results show a decrease in MCL1 expression and an increase of BTG2 and FBXW7 proteins in shPiwil1 mice tumors. Figure adapted from Huang et al. 2021.

The next paper I looked at was “Inhibition of Mcl-1 Promotes Senescence in Cancer Cells: Implications for Preventing Tumor Growth and Chemotherapy Resistance”. This paper uses different cell lines from human colon cancer patients to conduct trials on the effects of inhibiting MCL1. They looked at how the inhibition of MCL1 in these cell lines affected the senescence of cells. Since MCL1 is overexpressed due to PIWIL1 being overexpressed in GBMs, the inhibition of MCL1 has similar effects as the inhibition of PIWIL1. The researchers conducted an experiment using HCT116 vector cells and Mcl-1up cells. The authors overexpressed Mcl-1 by

transiently transfecting a vector overexpressing Mcl-1 into cell lines (Mcl-1up cells). To confirm Mcl-1 overexpression in the Mcl-1up cells, the researchers used western blotting to verify the increased levels of Mcl-1 protein in those cells. They used SA-β-gal staining to test the response of cells to doxorubicin, a chemotherapy drug. They treated normal and overexpressed Mcl-1 cells with or without doxorubicin for 6 days (Fig 4). They found that the cells with Mcl-1 overexpression did not undergo cellular senescence when treated with doxorubicin. This means that when Mcl-1 is overexpressed, cells are more likely to become cancerous. Next, the researchers focused on the number of progressive multifocal leukoencephalopathy (PML) bodies per nucleus in both HCT116 vector cells and Mcl-1up cells (Fig 5). PMLs are markers of various cellular processes such as senescence, cancer, and response to chemotherapy treatments. They conducted the experiment for 4 days with and without doxorubicin treatment and found that when Mcl-1 was overexpressed, the treatment was not as effective (Figs 4,5). The results showed that cells with reduced Mcl-1 levels had higher percentages of SA-β-gal-positive cells, indicating higher levels of senescence. This shows that reducing Mcl-1 levels in HCT116 cells allows for the start of chemotherapy-induced senescence[3].

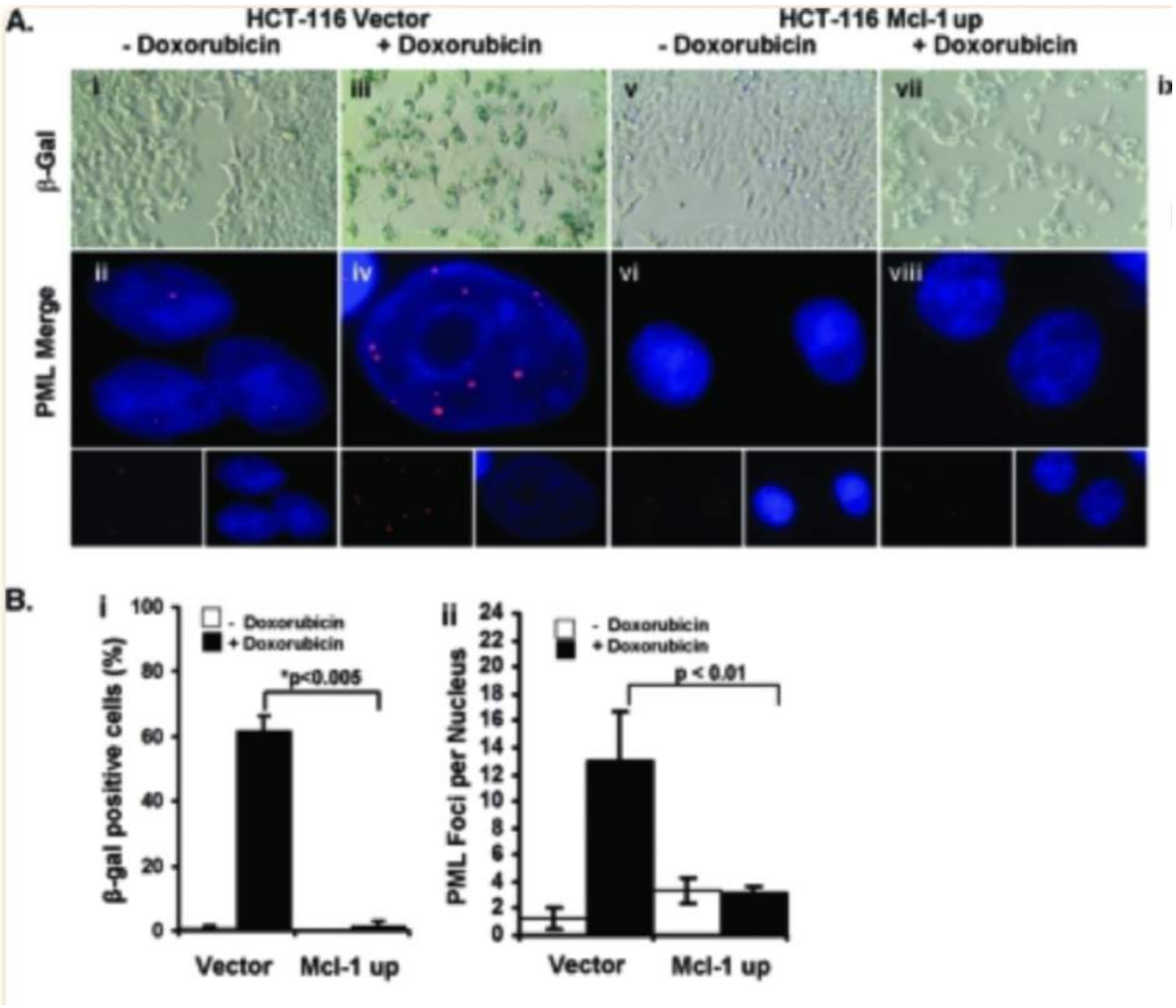

Figure 4. Cancer cells with increased Mcl-1 expression no longer respond to doxorubicin. **A.** Cells with normal and elevated Mcl-1 levels were grown with and without doxorubicin. These cells were stained with anti-PML antibody and a red secondary antibody to visualize the anti-PML antibody. Cells with increased Mcl-1 levels showed no increase in PML foci after doxorubicin treatment, however, cells with normal Mcl-1 levels did show an increase in PML foci. **B.** (i) Cells were stained with SA-β-gal. There is a significant decrease in B-gal positive cells in Mcl-1 up cells treated with doxorubicin compared to control cells treated with doxorubicin. (ii) There is an increase in PML foci per nucleus in the vector cells compared to Mcl-1 up cells with doxorubicin treatment. Figure adapted from Bolesta et al. 2012.

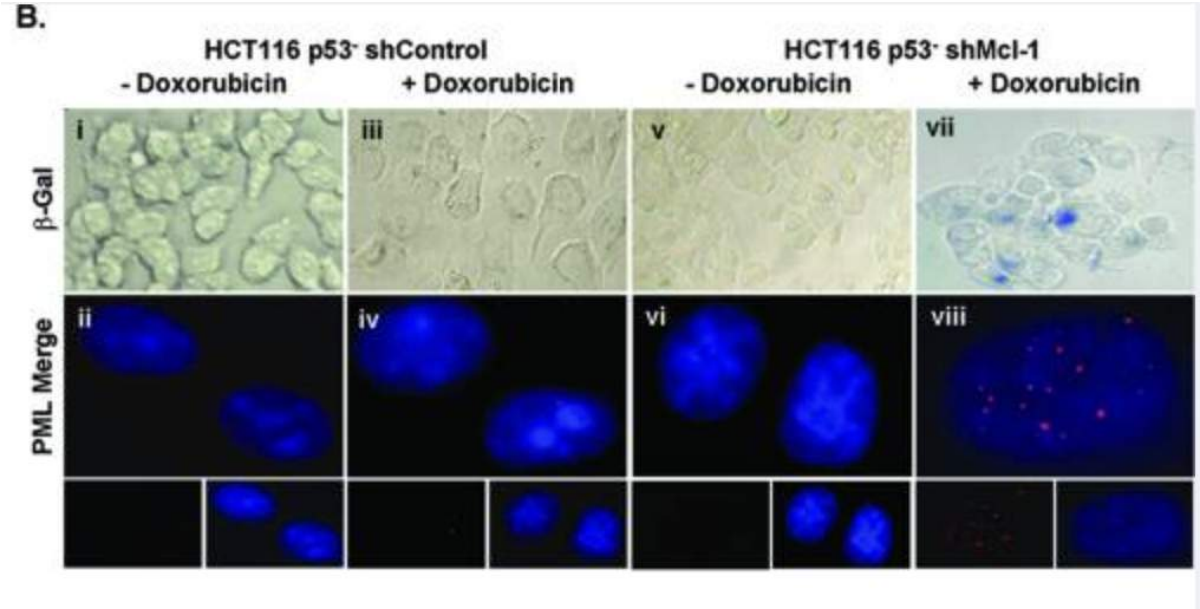

Figure 5. p53- cancer cells with a knockout of Mcl-1 respond positively to doxorubicin. B. Images show SA-β-gal+ cells in HCT116 p53− control cells and Mcl-1 knockout cells with and without doxorubicin treatment. Results show that p53- Mcl-1 knockout cells have an increase in PML foci compared to p53- control cells. Figure adapted from Bolesta et al. 2012.

Next, I analyzed a review, “The Regulation and Role of piRNAs and PIWI Proteins in Cancer”, related to my research question[4]. It is believed that when PIWI proteins are expressed in non-germ cells, they give these cells stem cell-like properties, potentially contributing to cancer development. A meta-analysis of multiple databases revealed that higher levels of PIWIL1 and lower levels of PIWIL4 are generally associated with increased malignancy in cancer. In glioblastomas, particularly in glioma stem cells, PIWIL1 is overexpressed. This protein regulates cell viability and self-renewal of these stem cells and is involved in mRNA stability. Knockdown of PIWIL1 reduces tumor growth and improves survival in mice with glioblastoma. There is a microRNA, miRNA-154-5p, that is downregulated in glioblastoma and targets PIWIL1 mRNA for degradation, acting as a tumor suppressor. This suggests that PIWIL1 has a regulatory role in glioblastoma development and the interactions with specific microRNAs may play a part in the tumorigenesis. One of the main roles of PIWI proteins is to produce PIWI-interacting RNAs

(piRNAs). piRNAs are involved in epigenetic silencing of transposons. In some cancers, certain PIWI proteins increase, however overall the number of piRNA molecules usually stay low. For pancreatic cancer, PIWIL1 becomes an oncogene even without an increase of piRNAs. Researchers are exploring if the oncogenic effects of PIWIL1 are lessened when there are higher amounts of piRNAs. piRNAs can interfere with important signaling that control gene expression. These piRNAs and their partner proteins, PIWI, can sometimes have aberrant functions in cancers[4]. Thus, these could be important targets in treating cancers. However, more information must be known about their interactions and functions within healthy and cancerous cells before being helpful cancer targets.

Section 4: Discussion

When PIWIL1 was knocked down, the size of GBM tumors decreased and the survival rate of the mice increased[2]. This shows that without PIWIL1, the tumor cannot survive. This data reinforces my hypothesis that elevated levels of PIWIL1 trigger the formation of gliomas. The tumor seems dependent on this irregular protein activity, suggesting that an initial overexpression of PIWIL1 might be necessary for the tumor's survival. The GSC's with knocked down PIWIL1 had sphere numbers nearing zero while the GSC's with PIWIL1 had sphere numbers in the 30's. Without PIWIL1 the tumor shrunk drastically, showing that it is dependent on the overexpression of that protein. In the transplanted mice xenografts, the survival rate plummeted to zero for the ones with PIWIL1 overexpression but were greater and stable for the mice with knocked down PIWIL1 expression. This also indicates that Piwil1 is essential for the survival of the tumor, as the rate of survival is higher when Piwil1 is absent. This increased rate suggests that the tumor is less prominent when Piwil1 is not present[2]. The second paper I looked at had similar results supporting my hypothesis. Since Mcl-1 is overexpressed due to the overexpression of Piwil1, it is another marker of whether the tumor will thrive or not. Doxorubicin, a chemotherapy drug, was not as effective in treating p53-cancer cells with increased MCL-1 expression. The overexpression of MCL-1 hindered the ability of the drug to effectively target the cancer cells compared to cancer cells with normal levels of MCL-1. The tumor requires overexpression of PIWIL1 and MCL-1 in order to grow and form. Without these genes and proteins, the tumor may be unable to form, supporting my hypothesis that PIWIL1 overexpression is necessary for glioma formation[3]. Although this cannot be said for sure without more research, the data from these papers points to my hypothesis being correct. More research on PIWIL1 expression before and after tumor formation needs to be done in order to better understand this concept.

Section 5: Conclusion

My research on this topic aimed to understand the complex relationship between the formation of gliomas and PIWI overexpression. The papers I reviewed suggest that elevated levels of PIWI proteins, specifically PIWIL1, are associated with glioma formation. The experiments I analyzed used glioma stem cells, mouse xenograft models, and cancer cell lines. Knocking down PIWIL1 resulted in decreased tumor size, increased survival rates, and reduced glioma malignancy. These results support my hypothesis that the overexpression of PIWIL1 plays a large role in glioma tumorigenesis. The overexpression of Mcl-1, a protein regulated by PIWIL1, was also shown to hinder the effectiveness of chemotherapy drugs. All of these results suggest that PIWIL1 plays a crucial role in glioma development but more research needs to be done in

order to be sure. Understanding the relationship between gliomas and PIWIL1 could be crucial in developing more targeted treatments and therapies for these cancers.

Section 6: References

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