Investigating Mdh1's Involvement In Suppressing Cell Proliferation In Mouse Gastrulation

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Studies have shown that the arginine methylation of MDH1 by CARM1 suppresses the cell proliferation of pancreatic cancer. MDH1, a gene crucial to metabolism and The Citric Acid Cycle, in part catalyzes an unconventional metabolic pathway that pancreatic cancer cells rely on for glutamine production, which is needed for cell proliferation. The arginine methylation of MDH1 by CARM1 inhibits MDH1 and leads to glutamine depletion, thus lowering the rate of pancreatic cancer cell proliferation.

This study delves to determine whether or not arginine methylation of MDH1 by CARM1, leading to glutamine depletion and suppressed cell proliferation, occurs as early as gastrulation. This was done by examining the gene expression of several genes of interest across gastrulation to find relationships between them. The expression of genes correlated to MDH1 inhibition, suppressed cell proliferation, and the arginine methylation of MDH1 were compared with the expression of MDH1 using violin plots and 2D scatter plots created with R. Certain relationships between the genes of interest and MDH1 would indicate that the arginine methylation of MDH1 is occurring as early as gastrulation.

This discovery could prove to be invaluable in the fight to treating pancreatic cancer patients, especially so in rare embryonic cases, and preventing future incidences of cancer in high-risk patients.

Introduction

Cancer continues to be a major cause of disease and death across the globe, resulting in nearly ten million deaths each year. In cancer patients, malignant cells divide uncontrollably and metastasize locally and to remote organs and tissues. The rate of cancer cell proliferation, or how quickly a cancer cell copies its DNA and divides into two cells determines the speed of progression of disease. Part of the survival strategy of cancer stem cells may be manifested by alterations in cell metabolism, which is key to cell proliferation (Feitelson et al., 2015).

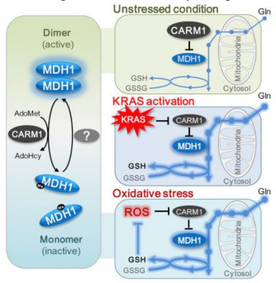
Alterations in cell metabolism may stimulate tumorigenesis, or the transformation of normal cells to malignant cells. However, studies have shown that investigating the genes involved in metabolism stimulating cell proliferation may provide insight into suppressing cancer cell proliferation.

Malate Dehydrogenase 1 (MDH1), an enzyme encoded by the MDH1 gene, is one of these genes critical to cell metabolism. MDH1 catalyzes the reversible oxidation of malate to oxaloacetate in the Citric Acid Cycle. Localized to the cytoplasm, MDH1 plays a crucial role in metabolic coordination between the cytosol and mitochondria.

Studies have shown that the arginine methylation of of MDH1 by protein arginine methyltransferase 4 (PRMT4/CARM1) represses mitochondrial respiration, inhibits glutamine utilization, reduces cellular NADPH level, and sensitizes cells to oxidative stress. PRMT4 has also been shown to inhibit MDH1 by disrupting its dimerization.

Pancreatic cancer cells rely on an unconventional metabolic pathway to rewire glutamine metabolism and produce NADPH. MDH1, along with aspartate aminotransferase and malic enzyme 1, catalyzes this pathway, meaning that the arginine methylation of MDH1 by CARM1 may inhibit glutamine metabolism and suppress pancreatic cancer (Wang et al., 2016).

The suppression of cell proliferation of pancreatic cancer in embryos has not been studied previously. Although cancer is rarely found in embryos, studying gene expression in mouse gastrulation may provide insight into MDH1 suppressing cancer cell proliferation later in life. This project aims to discover whether or not the inhibition of MDH1, and thus, the suppression of pancreatic cancer, can be seen as early as gastrulation, the embryonic stage in which the embryo changes from a blastula (containing a single layer of cells) to a gastrula (containing multiple layers of cells). The expression of certain genes was investigated in various mouse embryos throughout gastrulation using R. Specifically, signs of MDH1 being inhibited and the suppression of cell proliferation were studied. If successful, this study could contribute to the effort of preventing cancer as early as gastrulation.



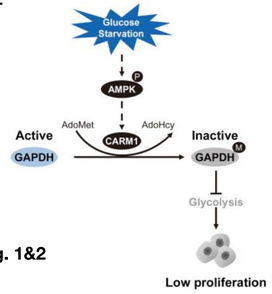


Fig. 1&2

Methods

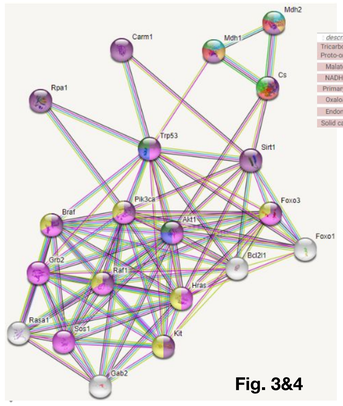
Along with a thorough review of relevant literature, the String Database (string-db.org) was utilized to identify several key genes of interest related to the inhibition of MDH1. Nearly all genes including several proto-oncogenes were involved in the primary metabolic process. Eventually, the following genes of interest were identified:

MDH1: an essential gene in metabolism that encodes an enzyme which catalyzes the NAD/NADH-dependent, reversible oxidation of malate to oxaloacetate in metabolic pathways like the citric acid cycle. In part catalyzes a metabolic pathway which pancreatic cancer cells depend on.

SIRT1: encodes the protein Sirtuin 1, which activates mitochondrial signals and pathways regulating the expression levels of genes (such as MDH1) crucial for proliferation and ATP generation (Hadar et al., 2017) - inhibited by the knockdown of MDH1.

PRMT4/CARM1: encodes the protein CARM1, which plays an important role in androgen receptors - methylates and inhibits MDH1 by disrupting its dimerization through arginine methylation.

mTORC1: encodes the protein mTORC1, which activates the translation of proteins - inhibition of mTORC1 leads to glutamine deprivation, so it can be used as an indicator of suppressed cell proliferation.



description	count in network	strength	false discovery rate
Tricarboxylic acid cycle	3 of 25	2.12	0.00028
Proto-oncogene	6 of 98	1.83	1.89e-07
Malate metabolic process	2 of 8	2.44	0.0021
NADH metabolic process	2 of 27	1.91	0.0118
Primary metabolic process	13 of 6369	0.35	0.0260
Oxaloacetate metabolic process	3 of 7	2.67	6.17e-05
Endometrial cancer	9 of 58	2.23	3.40e-16
Solid cancer cell	2 of 2	3.04	0.00058

Fig. 3&4

Finding Evidence of MDH1 Knockdown:

The relationship and expression of these genes was studied by extracting information from the database using R. Certain patterns between the genes of interest serve as evidence that MDH1 is being inhibited. For example, as SIRT1 regulates the expression of MDH1, a direct relationship between the expression of these two genes would indicate that SIRT1 is being inhibited because of the knockdown of MDH1. Furthermore, CARM1 and MDH1 should have an inverse relationship, as CARM1 inhibits MDH1. When CARM1 expression increases, MDH1 expression should decrease. Various 2D scatter plots, UMAPs, and violin plots were generated in RStudio using data collected from embryos (E6.5-E8.5 days) in gastrulation. All of the genes of interest were very widely expressed as they are involved in metabolism, a process crucial to the survival of a cell, so the cell type was only slightly taken into consideration.

Finding Evidence of Suppressed Cell Proliferation:

As the database does not contain embryos with pancreatic cancer, resources are too limited to find direct evidence of cell proliferation. Thus, another method to look at signs of suppressed cell proliferation needed to be found.

Research has demonstrated that pancreatic cancer cells are sensitive to glutamine (Gln) deprivation (Guillaumond et al., 2013, Ying et al., 2012). Most cells utilize Gln to fuel the The Citric Acid Cycle, however, oncogenic activation of KRAS2 in PDAC cells repurposed Gln through a distinct pathway in which Gln-derived aspartate was transported into the cytoplasm. This pathway (discussed in the Introduction) is catalyzed by MDH1, and a decrease in MDH1 leads to glutamine deprivation. Glutamine deprivation is directly associated with suppressed cell proliferation, and thus can be used as an indicator for it.

Inhibition of the mTORC1 gene leads to glutamine depletion (Fan et al., 2020). The expression of mTORC1 was compared to the expression of the genes of interest in hopes of finding evidence of glutamine depletion and suppressed cell proliferation as a result of the arginine methylation of MDH1 by CARM1 in gastrulation.

Results

Evidence of MDH1 Knockdown:

Various different options were examined in R Studio to look for relationships between genes of interest over gastrulation. Originally, violin plots were chosen as the ideal method to compare gene expression. However, as many of the genes of interest were expressed widely across cell types, violin plots were difficult to analyze.

The violin plots collected are shown below (endodermal cell type), however, these plots were difficult to analyze.

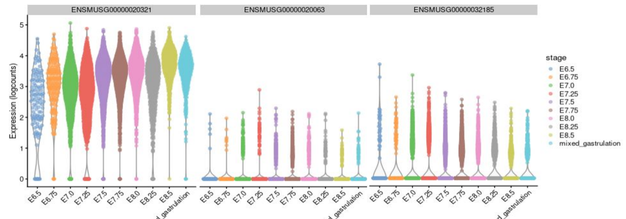


Fig. 5, 6, & 7: MDH1 on the left, SIRT1 in the middle, CARM1 on the right

After creating these violin plots, there was still insufficient evidence to effectively support a relationship between MDH1 and the genes of interest. MDH1 was virtually evenly expressed, so it was difficult to determine how gene expression changed throughout gastrulation. Furthermore, SIRT1 and CARM1 did not show a definitive pattern when changing gene expression across gastrulation. 2D Scatter plots, shown below, demonstrated a relationship between the expression of genes in a much clearer fashion.

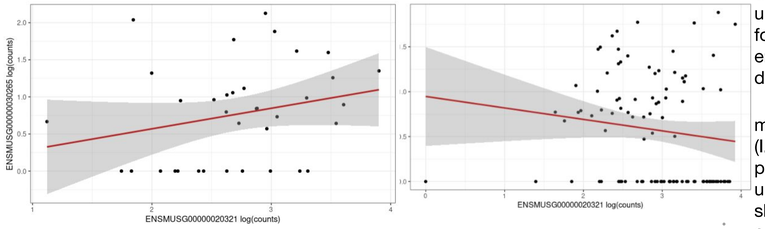


Fig. 8: MDH1 X-axis, SIRT1 Y-Axis

Fig. 9: MDH1 X-axis, CARM1 Y-Axis

Fig. 8: The best-fit line, along with the points showing gene expression, clearly show a direct relationship between MDH1 and SIRT1. The inhibition of MDH1 and the inhibition of SIRT1 should be directly correlated, and the graph supports this. As the expression of MDH1 increases, so does the expression of SIRT1. The graph clearly demonstrates a direct relationship between SIRT1 and MDH1, indicating that SIRT1 is acting as an indicator of MDH1 expression levels. However, the plot does not provide evidence to indicate that MDH1 knockdown is occurring.

Fig. 9: An inverse relationship between MDH1 and CARM1 is clearly demonstrated by the graph. The arginine methylation of MDH1 by CARM1 leads to the inhibition of MDH1, so a decrease in CARM1 expression should lead to an increase in MDH1 expression, and vice versa. As MDH1 levels increase the best-fit line clearly demonstrates a decrease in CARM1, indicating that CARM1 is causing the knockdown of MDH1.

Both of these 2D scatter plots serve as evidence that the knockdown of MDH1 is occurring. SIRT1 is being inhibited, which studies have shown to be directly correlated with MDH1 knockdown. Moreover, CARM1 and MDH1 expression counts have an inverse relationship, demonstrating that the expression of CARM1 is causing MDH1 inhibition.

Evidence of Suppressed Cell Proliferation:

As it is currently not possible to look at pancreatic cancer cells in gastrulation with the dataset, the mTORC1 gene was used as an indicator of cell proliferation. Inhibition of mTORC1 is directly associated with glutamine deprivation, which is directly associated with suppressed cell proliferation. Thus, a direct correlation between the expression of mTORC1 and MDH1 would serve as evidence of suppressed cell proliferation.

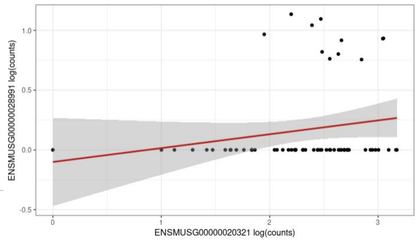


Fig. 10: MDH1 X-axis, mTORC1 Y-Axis

Fig. 10: Not much coexpression is displayed between mTORC1 and MDH1, however, the best fit line does seem to show a slight correlation (direct relationship) between MDH1 and mTORC1. As the expression of mTORC1 increases, so does the expression of MDH1, indicating that mTORC1 may be indicating increased cell proliferation because of increased MDH1 expression.

Since the 2D plots show that CARM1 is inhibiting MDH1 and that MDH1 is directly correlated to cell proliferation, evidence suggests that the process of suppressed cell proliferation *is* occurring as early as gastrulation.

Discussion

Examining the violin and scatter plots from RStudio showed that:

- The expression of SIRT1 is directly correlated with higher MDH1 levels, suggesting that low levels of SIRT1 does serve as an accurate indicator of MDH1 knockdown.
- The expression of CARM1 is associated with lower MDH1 levels, suggesting that the arginine methylation of MDH1 by CARM1 is occurring in gastrulation.
- mTORC1 and MDH1 are directly related, suggesting that MDH1 levels are directly responsible for cell proliferation rates, which are being lowered by the presence of CARM1.

Analyzing the plots provided favorable results, however, a limitation of the project was that the correlations between each gene may have been affected by unknown factors. Although evidence suggests the relationships discovered between the expressions of these genes is a direct result of the arginine methylation of MDH1 by CARM1, it is unknown whether or not this process is the sole reason for what was found. Furthermore, all plots of gene expression were made using the endothelial cell type - results may vary when expression is looked at in different cell types.

Other than proving the hypothesis, results solidified claims about many of the genes of interest already discussed in relevant literature (I.E. SIRT1 regulates the expression levels of genes). The effect of this process in suppressing pancreatic cancer in children or embryos is unable to be studied with current resources, however, literature has shown the process of MDH1 knockdown to suppress pancreatic cancer cell proliferation in adult patients.

Conclusion

The research conducted suggests that CARM1 is critical to regulating the metabolism of pancreatic cancer via MDH1 methylation, affirming claims of this theory already found in scientific literature. More importantly, evidence shows that cell proliferation is already occurring as early as gastrulation. This discovery could prove to be invaluable in the fight to treating pancreatic cancer patients, especially so in rare embryonic cases. Currently, development of selective and potent CARM1 activators to fight cancer is under intensive study (Zeng et al., 2013). Moreover, more research conducted on this process specifically in embryos could potentially provide insight into how to prevent future cases of pancreatic cancer.

In the future, gene expression could be studied in a small cohort of embryos with cancer. Furthermore, the specific phases of gastrulation, or in what phase of gastrulation does MDH1 knockdown most occur, could be studied. Current literature and this study only look into suppressing cell proliferation of specific age groups - a wider demographic should be studied, and results should also be compared across various demographics.

References

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