Protein Arginine Methyltransferase 5 as a Therapeutic Target for *KRAS* Mutated Colorectal Cancer

Thesis Submitted in Partial Fulfillment of the Requirements of the Jay and Jeanie Schottenstein Honors Program

Department of Biology, Yeshiva College, Yeshiva University

May 2020

David Shifteh

Mentor: Radhashree Maitra, Ph.D.

Associate Professor of Biology

Table of Contents

Abstract

Colorectal cancer (CRC) is the third most commonly occurring cancer globally. One of the current therapies used in CRC treatment is the anti-epidermal growth factor receptor (anti-EGFR) monoclonal antibody, which is commercially marketed as cetuximab. However, cetuximab has been seen to be detrimental for patients who have CRC with a mutated *KRAS* gene.

The *RAS* signaling pathway is involved in many cellular processes including cell proliferation. A driver mutation in the *KRAS* gene causes the protein to become overactive and drives abnormal cell growth and proliferation. Nearly 45% of colorectal cancer (CRC) patients harbor a mutation in their *KRAS* gene, for which despite many years of research, there are still no drugs available. Other treatments that indirectly target mutant *KRAS* are thus highly sought after and much needed.

Protein Arginine Methyltransferase 5 (PRMT5) is a transcription regulator for multiple cellular processes that is currently being tested as a potential target/biomarker in several cancer types. PRMT5 has been found to be over-expressed in various cancers, and PRMT5 over-expression is also correlated with increased cell growth and decreased patient survival.

Herein we have found that not only do *KRAS* mutant CRC cells respond effectively to PRMT5 inhibitor treatment, but that *KRAS* mutant CRC cells show an even greater degree of inhibition, apoptosis, and cell cycle arrest when compared to their *KRAS* wild type (WT) counterparts after being treated with PRMT5 inhibitor.

Introduction

Colorectal cancer (CRC) is the second largest cause of cancer death in the US [1]. It is estimated that in 2020 there will be 147,950 new diagnoses and 53,200 new deaths of colorectal cancer in the United States alone [1]. Most cases of CRC take over 10 years to fully develop and advance through the adenoma-carcinoma sequence [2]. Inactivating mutations of the *APC* tumor suppressor gene are an early step in the process and occur in over 70% of colorectal adenoma [3]. Additional activating mutations of the *KRAS* oncogene and inactivating mutations of the *TP53* tumor suppressor gene further promote the adenoma-carcinoma sequence [3].

Epidermal growth factor receptor (EGFR) is a transmembrane receptor tyrosine kinase (RTK) that is responsible for sending a downstream signal to initiate cellular division. When EGFR is over-expressed, excessive cellular proliferation results [4]. In CRC, EGFR is estimated to be significantly over-expressed in 60%-80% of tumors, and EGFR over-expression is associated with a poor prognosis [5]. Cetuximab—a monoclonal antibody which binds to the extracellular domain of EGFR and prevents ligands from binding—was thus developed as a treatment for CRC [6].

Cetuximab however, has been found to have two key limitations. First, cetuximab only works in cells that do not have a mutation in a gene that codes for a protein that is downstream of EGFR—such as the *KRAS* gene [7]. Second, many patients who at first do respond to cetuximab treatment, eventually develop resistance to cetuximab due to secondary mutations, which later develop downstream of EGFR [8]. In some cases, cetuximab has even been seen to be detrimental for patients who have CRC with a mutated *KRAS* gene [9].

KRAS is a membrane bound GTPase that plays an early and lead role in many signal transduction pathways [10]. Activating mutations in *KRAS* have been shown to result in increased cellular proliferation as well as suppression of apoptosis [11, 12]. As *KRAS* has been determined to be mutated in nearly 45% of CRC patients, much research has been pursued in order to develop an inhibitor for mutant *KRAS* [13]. However, despite many years of research, there are still no therapies available that target mutant *KRAS* in CRC [14]. Other treatments that indirectly target mutant *KRAS* are thus urgently sought after in the clinical setting.

Arginine methylation is an important post-translational modification which functions as an epigenetic regulator of transcription, in addition to playing key roles in pre-mRNA splicing, DNA damage signaling, and cell signaling [15]. Cancer is progressively being seen to not only result from genetic mutations, but also from epigenetic alterations [16]. Increasing evidence is indicating that protein arginine methyltransferases (PRMT) play an important role in cancer progression and maintenance [17].

Protein arginine methyltransferase 5 (PRMT5) is an enzyme which catalyzes the methylation of arginine residues on target proteins [18]. PRMT5 has been found to specifically regulate gene expression in two major ways: the methylation of histones, and the methylation of key transcription factors such as p53 and E2F1 [18]. It has been reported that PRMT5 is over-expressed in approximately 75% of CRC cases [17].

Fibroblast growth factor receptor 3 (FGFR3) is a protein that has been shown to be both over-expressed in CRC, and to promote tumor growth [17]. PRMT5 has been found to play a

role in regulating FGFR3 by binding to the FGFR3 binding site within the promoter region, and thereby enhancing FGFR3's transcription [17].

Furthermore, PRMT5 has also been seen to play a profound role in regulating the downstream targets of FGFR3 such as AKT, ERK and mTOR [17]. Upon silencing PRMT5 in CRC cells, FGFR3 expression has been found to be reduced, which thus leads to the reduced expression of AKT, ERK, as well as mTOR [17]. These findings suggest that FGFR3 over-expression in CRC may actually be a result of the over-expression of PRMT5.

Additionally, PRMT5 has also been found to antagonize several pro-apoptotic signaling pathways [19]. The tumor necrosis factors (TNF)-related apoptosis-inducing ligand (TRAIL) binds to death receptors DR4 and DR5 and induces apoptosis. PRMT5 has been found to physically interact with DR4 and DR5, and to inhibit the apoptotic effect of TRAIL [19].

Moreover, PRMT5 has been shown to methylate E2F-1 transcription factor, and thus inhibit E2F-1's ability to induce apoptosis [20]. PRMT5 depletion has also been seen to increase E2F-1's expression levels, causing increased apoptosis and a reduction in cellular proliferation [20].

By contrast, inhibition of PRMT5 by AMI-1, or knockdown of PRMT5 by shRNA, has been shown to restore key regulatory pathways which are involved in cell growth, survival, migration, and tumor suppressor activity [17].

The above examples clearly show that PRMT5's regulatory activities are largely pro-proliferation and pro-survival, and thus a PRMT5 inhibitor therapy can be potentially used as a new treatment for *KRAS* mutated CRC.

Materials and Methods

Cell Culture

Six CRC cell lines: HCT116, SW620, CaCo2, HT29, HKE3, and LIM2405, as well as one normal colon cell line: CCD841 were purchased from the American Type Culture Collection and cultured for use in this study. HCT116 and SW620 are *KRAS* mutant cell lines, whereas CaCo2, HT29, HKE3, and LIM2405, are *KRAS* WT cell lines. HCT116 and HKE3 are isogenic cell lines—HCT116 differs from HKE3 only by having an activating *KRAS* mutation. The cell lines were grown in media which contained 87% MEM, 10% FBS, 1% NEAA, and 2% HEPES buffer. The cells were passaged twice a week.

Quantitative Polymerase Chain Reaction (qPCR)

HCT116, SW620, CaCo2, HT29, and CCD841 were cultured, and then spun down into cell pellets. RNA was then extracted from the cell pellets using the Invitrogen™ PureLink™ RNA Mini Kit (Catalog #: 12183018A) as per the manufacturer's protocol. The concentration of the extracted RNA was then quantified using an Invitrogen™ Qubit™ RNA BR Assay Kit (Catalog #: Q10211) as per the manufacturer's protocol. The RNA was then converted into cDNA using the Maxima™ H Minus cDNA Synthesis Master Mix (Catalog #: M1661) as per the manufacturer's protocol. A T100™ Thermal Cycler was used to run the reaction. Next, qPCR was performed using the Applied Biosystems™ PowerUp™ SYBR™ Green Master Mix (Catalog #: A25918) as per the manufacturer's protocol. An Applied Biosystems 7300 Real-Time PCR System was used to run the qPCR. The primers used were purchased from sigma with the following sequences.

PRMT5 Forward Primer: 5'GTTCTGCTATTCATAACCCCA3'

PRMT5 Reverse Primer: 5'AATCCAGCACTAATTCCTCA3'

GAPDH Forward Primer: 5'AGGTGGAGGAGTGGGTGTCGCTGTT3'

GAPDH Reverse Primer: 5'CCGGGAAACTGTGGCGTGATGGCAA3'

Western Blot Analysis

HCT116, SW620, CaCo2, HT29, and CCD841 were cultured, and then spun into cell pellets. Total protein was then extracted from the cell pellets by freeze-thawing the cell pellets five times in liquid nitrogen for 30 seconds each time. The concentration of the extracted protein was then quantified using an Invitrogen™ Qubit™ Protein Assay Kit (Catalog #: Q33212) as per the manufacturer's protocol. A western blot was then performed using the Invitrogen™ Western Devices Benchtop Bundle (Catalog #: IW3000S) as per the manufacturer's protocol. The primary antibody used for PRMT5 detection was the Invitrogen PRMT5 Recombinant Rabbit Monoclonal Antibody (ST51-06) (Catalog #: MA5-32160). The secondary antibody used for PRMT5 detection was the Invitrogen Goat anti-Rabbit IgG (H+L) Secondary Antibody, HRP (Catalog #: 31460). Beta-actin, as well as the Invitrogen™ No-Stain™ Protein Labeling Reagent (Catalog #: A44449) was used for normalization. A ChemiDoc™ MP Imaging System was used for blot imaging.

PrestoBlue™ HS Cell Viability Assay

HCT116, SW620, HKE3 and LIM2405 cell lines were cultured in 96-well plates. 24 hours later the plates were treated with 1uM and 10uM concentrations of PRMT5 inhibitor (EPZ015666) and then incubated for 60 hours in a 37°C/5% CO_2 incubator. Invitrogen™ PrestoBlue™ HS Cell

Viability Reagent (Catalog #: P50200) was then added to the plates, and the plates were then incubated for 2 hours in a 37°C/5% CO_2 incubator. Fluorescence readings were then measured in a Beckman Coulter DTX 880 Multimode Detector plate reader at fluorescence excitation 535nm and emission 595nm wavelengths as per the manufacturer's protocol.

Guava® Annexin Red Kit Assay.

HCT116, SW620, HKE3 and LIM2405 cell lines were cultured in 6-well plates. 24 hours later the plates were treated with a 10uM concentration of PRMT5 inhibitor (EPZ015666) and then incubated for 60 hours in a 37°C/5% CO_2 incubator. An annexin assay was then performed using The Guava® Annexin Red Kit (Catalog #: FCCH100108) as per the manufacturer's protocol. The samples were run in a Guava® EasyCyte™ mini.

Guava® Cell Cycle Assay.

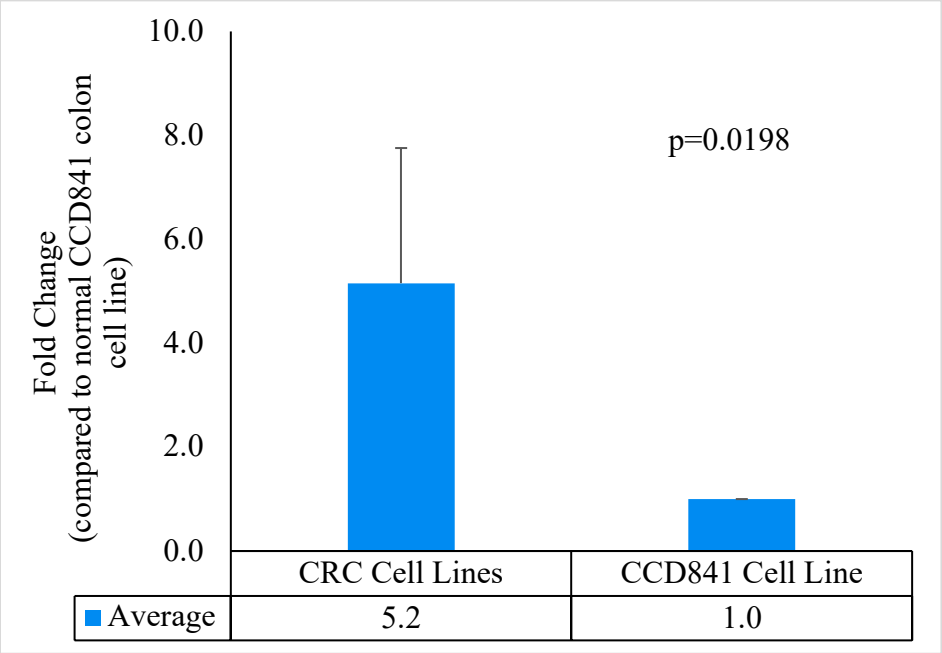
HCT116, SW620, HKE3 and LIM2405 cell lines were cultured in 6 well plates. 24 hours later the plates were treated with a 10uM concentration of PRMT5 inhibitor (EPZ015666) and then incubated for 60 hours in a 37°C/5% CO_2 incubator. A cell cycle assay was performed using The Guava® Cell Cycle Reagent (Catalog #: 4500-0220) as per the manufacturer's protocol. The samples were run in a Guava® EasyCyte™ mini.

Results

PRMT5 mRNA is over-expressed in CRC cells

A qPCR Assay was first performed to demonstrate whether PRMT5 is over-expressed at the transcriptional level in CRC cells. Our results show that PRMT5 is over-expressed in CRC cells in general by 5.2-Fold (p=0.0198) when compared to normal colon CCD841 cell line. More specifically, PRMT5 is over-expressed in *KRAS* mutant CRC cells by 7.0-Fold (p=0.00599), and PRMT5 is over-expressed in *KRAS* WT CRC cells by 3.7-Fold (p=0.0355). When comparing the PRMT5 expression of *KRAS* mutant CRC cells directly to *KRAS* WT CRC cells, it was seen that *KRAS* mutant CRC cells have a 1.9-Fold greater over-expression of PRMT5 (p=0.0256) (n=3) [Figure 1 a & b].

a



b

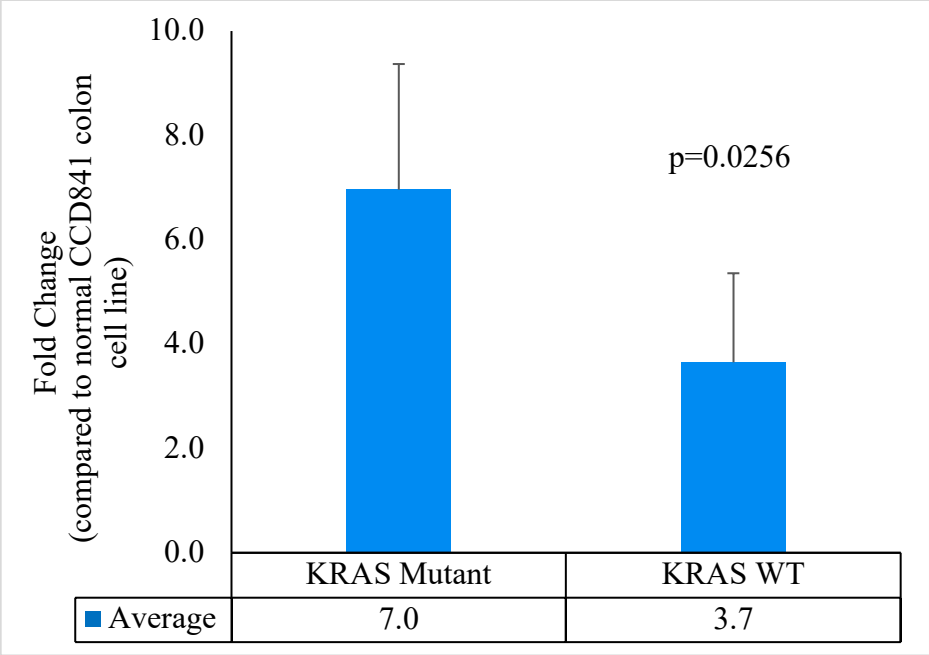


Fig. 1 PRMT5 is seen to be over-expressed in CRC cells at the transcriptional level by qPCR Analysis **a** PRMT5 is 5.2-Fold over-expressed in CRC cells when compared to normal colon CCD841 cells **b** PRMT5 is 1.9-Fold further over-expressed in *KRAS* mutant CRC cells when compared to *KRAS* WT CRC cells

PRMT5 protein is over-expressed in KRAS mutant cells

Western Blot Analysis was then performed to ascertain whether this over-expression of PRMT5 at the transcriptional level in CRC cells effected an over-expression of PRMT5 at the translational level in CRC cells. While our data showed that PRMT5 is 6.1-Fold (p=0.119) over-expressed in CRC cells in general, when compared to normal colon CCD841 cell line, the results do not meet significance. Similarly, our data shows that while PRMT5 is 2.7-Fold (p=0.191) over-expressed in *KRAS* WT CRC cells, the results do not meet significance. However, when looking specifically at PRMT5 expression in *KRAS* mutant CRC cells, our results show that

PRMT5 does indeed show over-expression by 11.3-Fold (p=0.00439). Moreover, when comparing the PRMT5 expression of *KRAS* mutant CRC cells directly to *KRAS* WT CRC cells, it was further seen that *KRAS* mutant CRC cells have a 4.2-Fold greater over-expression of PRMT5 (p=0.00107) (n=3) [Figure 2 a & b].

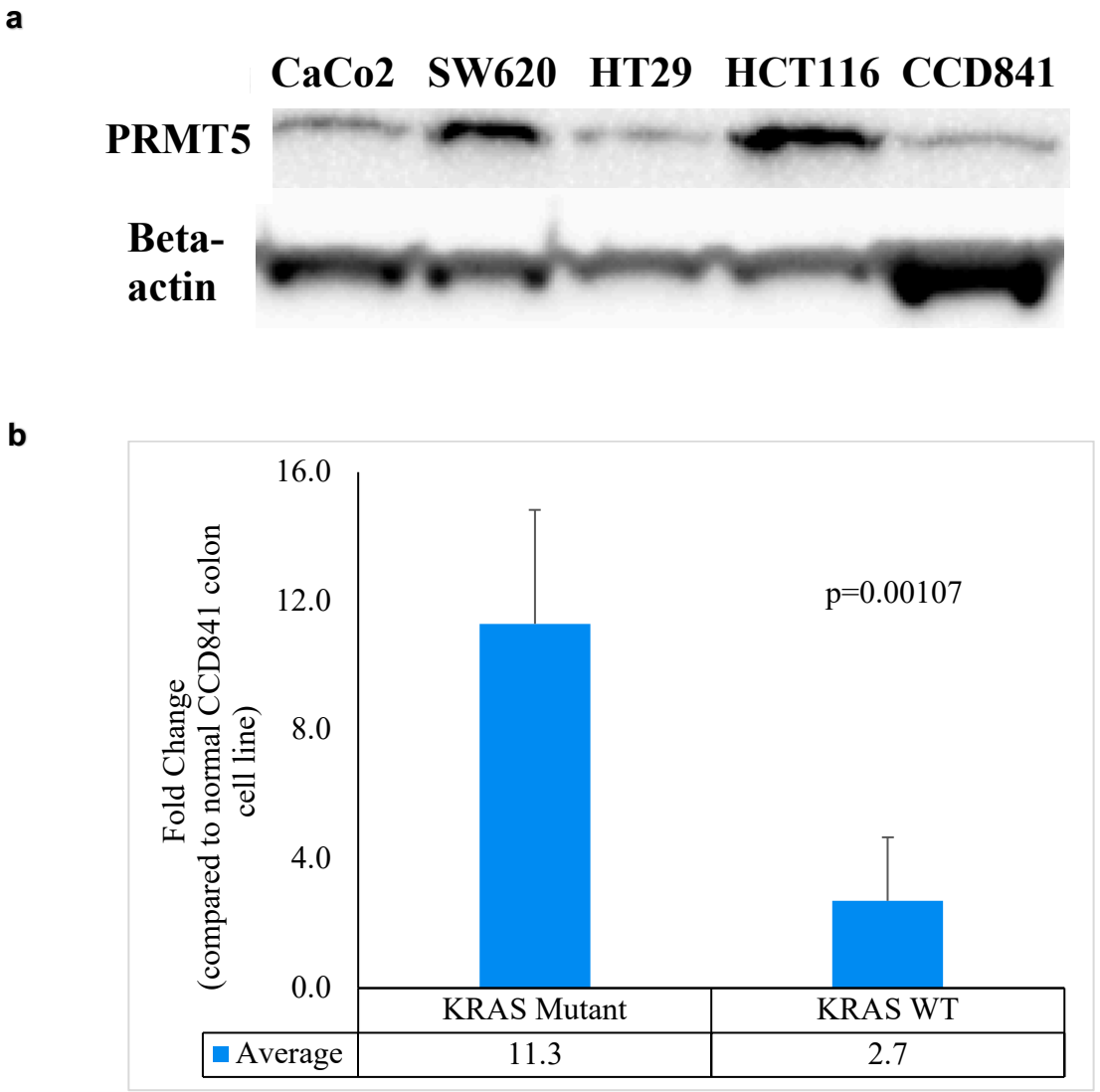
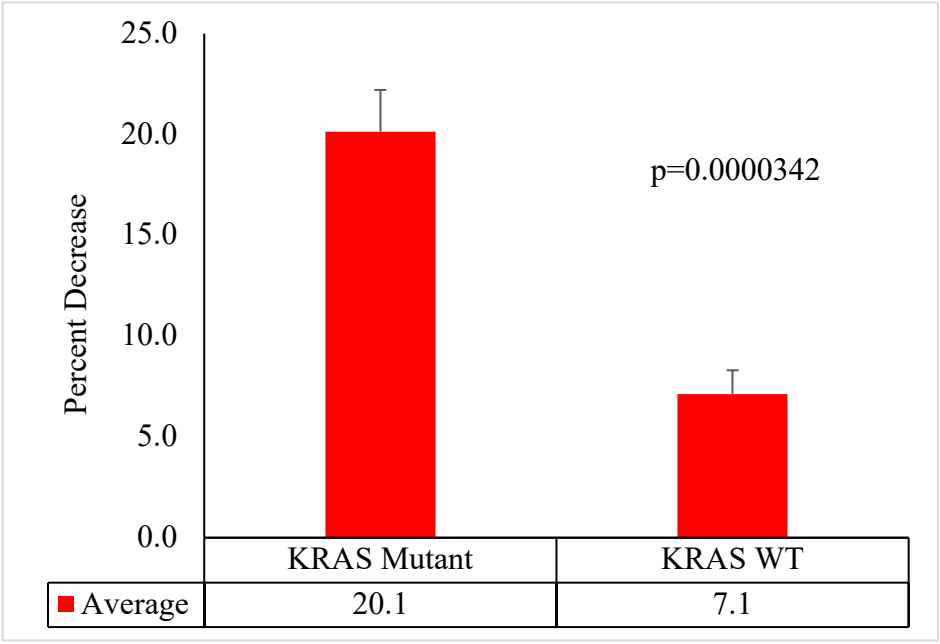


Fig. 2 PRMT5 is seen to be over-expressed in *KRAS* mutant CRC cells at the translational level by Western Blot Analysis. **a** Western blot results showing PRMT5 and Beta-actin bands **b** PRMT5 is 4.2-Fold further over-expressed in *KRAS* mutant CRC cells when compared to *KRAS* WT CRC cells

Inhibiting PRMT5 lowers cell viability in KRAS mutant cells

We next sought to determine whether this further over-expression of PRMT5 in *KRAS* mutant CRC cells has clinical applicability. Our PrestoBlue™ HS Cell Viability Assay data indicated that the further over-expression of PRMT5 observed in the *KRAS* mutant CRC cells resulted in a 2.8-fold and 2.0 fold (p=0.0000342, & p=0.00452, respectively) decrease in metabolic activity at 1 µM & 10µM PRMT5 inhibitor concentrations, respectively, in the *KRAS* mutant CRC cells when compared to the *KRAS* WT CRC cells (n=3) [Figure 3 a & b].

a



b

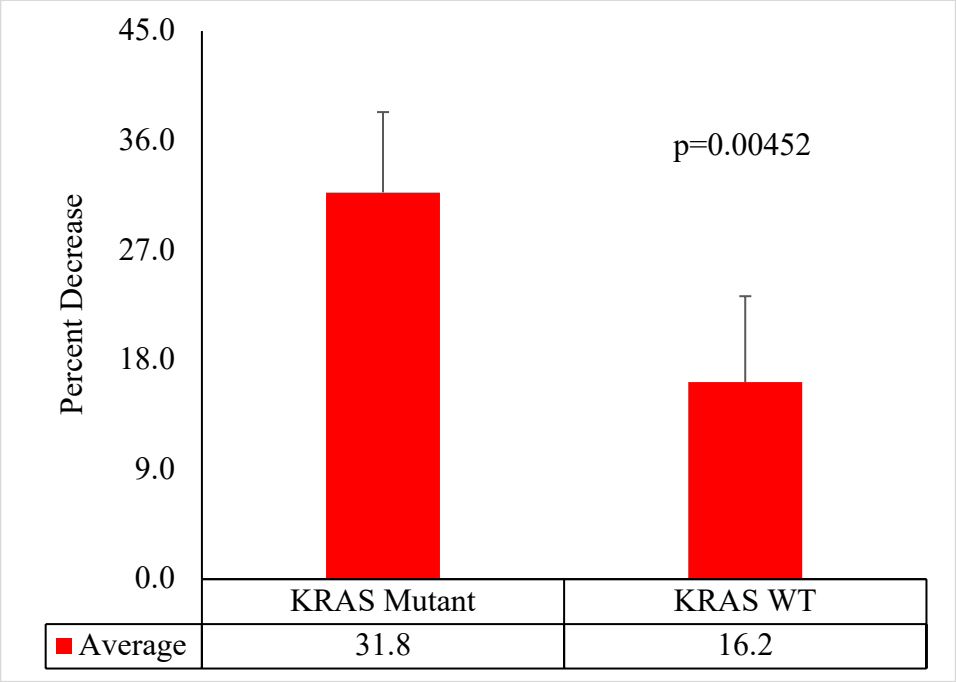
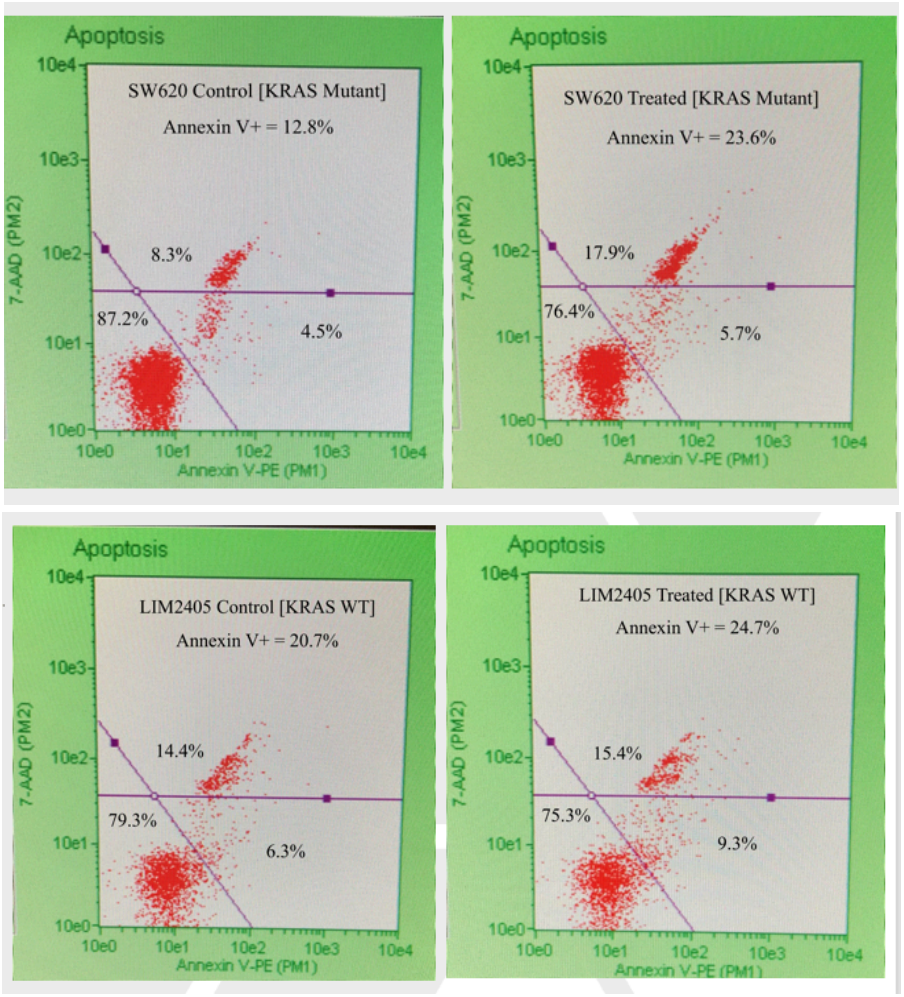


Fig. 3 PRMT5 inhibitor treatment showed a significant reduction in cell viability in *KRAS* mutant CRC cells when compared to *KRAS* WT CRC cells post 60 hours of PRMT5 inhibitor treatment by High-Throughput Presto Blue™ HS Cell Viability Assay. **a** 1µM PRMT5 Inhibitor treatment showed a further 2.8-Fold inhibition in *KRAS* mutant CRC cells **b** 10µM PRMT5 Inhibitor treatment showed a further 2.0-Fold inhibition in *KRAS* mutant CRC cells

PRMT5 inhibition stimulates apoptosis in KRAS mutant cells

We then examined whether part of the decrease in metabolic activity seen previously was due to cell death or controlled apoptosis. A Guava® Annexin Red Assay was therefore performed. A 5.9-fold ($p = 0.0470$) increase in apoptosis was seen in the *KRAS* mutant CRC cells when compared to the *KRAS* WT CRC cells post 60 hours of 10µM PRMT5 inhibitor treatment via Guava® Annexin Red Kit Assay (n=4) [Figure 4 a & b].

a


Apoptosis
SW620 Control [KRAS Mutant]
Annexin V+ = 12.8%
8.3%
87.2%
4.5%
Apoptosis
SW620 Treated [KRAS Mutant]
Annexin V+ = 23.6%
17.9%
76.4%
5.7%
Apoptosis
LIM2405 Control [KRAS WT]
Annexin V+ = 20.7%
14.4%
79.3%
6.3%
Apoptosis
LIM2405 Treated [KRAS WT]
Annexin V+ = 24.7%
15.4%
75.3%
9.3%
7-AAD (PM2)
Annexin V-PE (PM1)
10e0
10e1
10e2
10e3
10e4

b

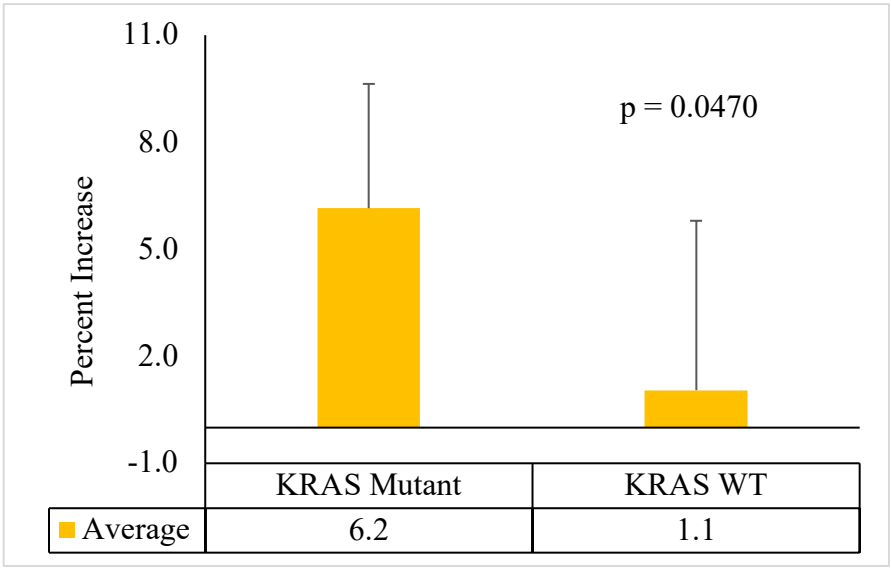
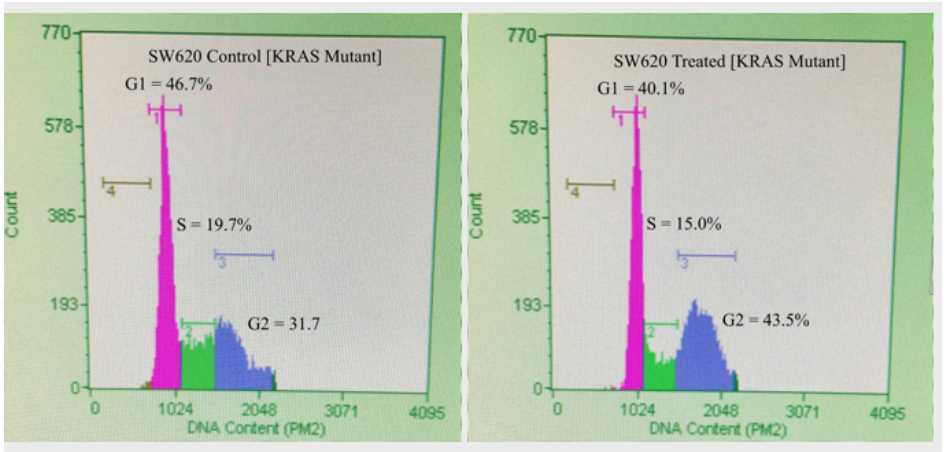

Percent Increase
11.0
8.0
5.0
2.0
-1.0
p = 0.0470
KRAS Mutant
KRAS WT
Average
6.2
1.1

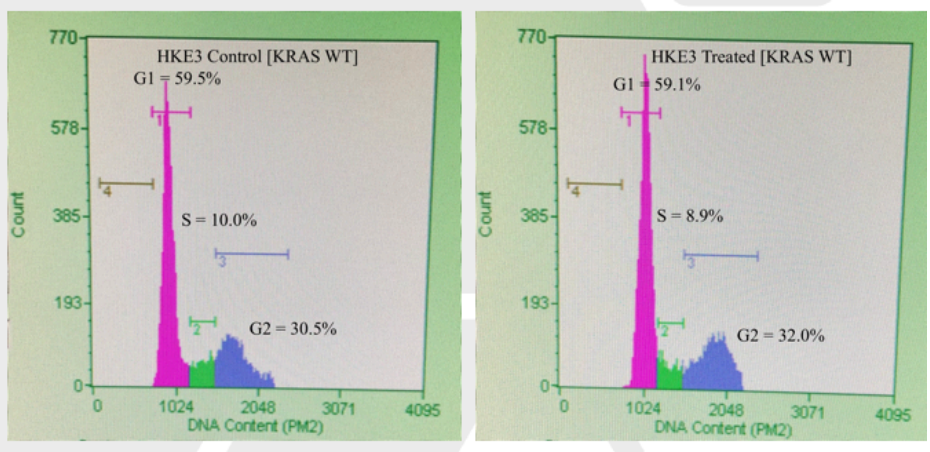
Fig. 4 PRMT5 inhibitor treatment showed a significant increase in apoptosis in *KRAS* mutant CRC cells when compared to *KRAS* WT CRC cells post 60 hours of 10µM PRMT5 inhibitor treatment by Guava® Annexin Red Kit Assay **a** Flow cytometry dot plots displaying annexin assay results **b** A 5.9-fold increase in apoptosis is seen in the *KRAS* mutant CRC cells

Inhibiting PRMT5 triggers G2 Phase arrest in KRAS mutant cells

Finally, a Cell Cycle Assay was then performed to determine whether any of the decrease in metabolic activity seen above was due to cells fixed in cell cycle arrest. Our results documented that a 9.2% increase ($p = 0.0201$) in G2 phase cell cycle arrest was seen in the *KRAS* mutant CRC cells when compared to the *KRAS* WT CRC cells post 60 hours of 10µM PRMT5 inhibitor treatment via Guava® Cell Cycle Assay (n=4) [Figure 5 a & b].

a





b

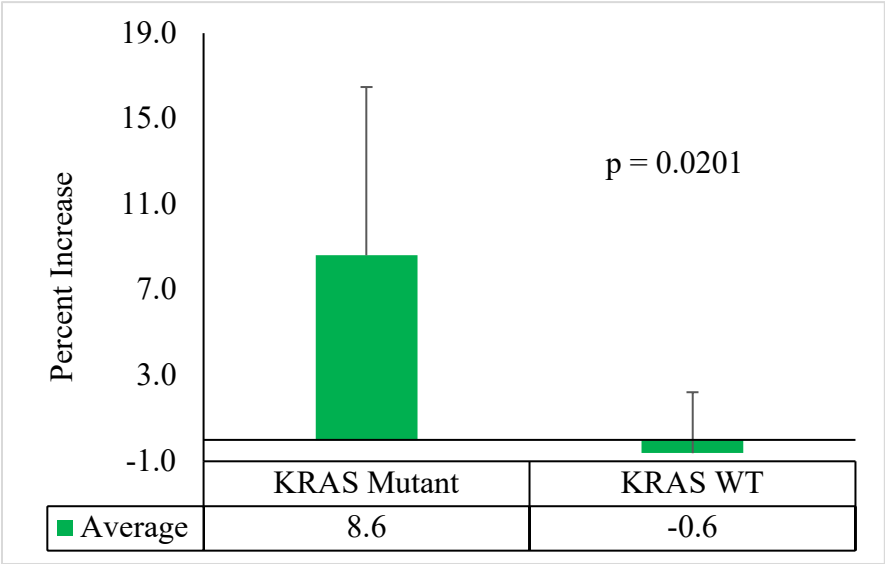


Fig. 5 PRMT5 inhibitor treatment showed a significant increase in G2 phase cell cycle arrest in *KRAS* mutant CRC cells when compared to *KRAS* WT CRC cells post 60 hours of 10µM PRMT5 inhibitor treatment by Guava® Cell Cycle Assay **a** Flow cytometry dot plots displaying cell cycle assay results **b** A 9.2% increase in G2 phase cell cycle arrest is seen in the *KRAS* mutant CRC cells

Discussion

As nearly 45% of CRC patients have a *KRAS* mutation, for which no targeted therapy is currently available, we performed this study to determine whether PRMT5, a transcription regulator for multiple cellular processes, could show efficacy towards treating *KRAS* mutant CRC [13, 18].

We first investigated whether PRMT5 is indeed over-expressed at the transcriptional level in CRC cells. Our qPCR data showed that not only is PRMT5 mRNA over-expressed in CRC cells in general, but that PRMT5 mRNA is further over-expressed in *KRAS* mutant CRC cells when compared to *KRAS* WT CRC cells. This data therefore demonstrates that *KRAS* mutant CRC cells produce a significantly higher amount of PRMT5 mRNA compared to *KRAS* WT CRC cells.

Upon determining that PRMT5 is over-expressed in CRC cells at the transcriptional level by qPCR, a Western Blot Assay was then performed to assess whether this additional PRMT5 mRNA that is synthesized in CRC cells is then translated to produce excess PRMT5 protein. Our Western Blot data established that PRMT5 expression at the translational level was significantly upregulated in *KRAS* mutant CRC cells when compared to *KRAS* WT CRC cells. This data thus corroborated the results of the qPCR and showed that PRMT5 is over-expressed at both the transcriptional and translational levels in *KRAS* mutant CRC cells when compared to *KRAS* WT CRC cells.

After verifying that PRMT5 is over-expressed at both the transcriptional and translational levels in *KRAS* mutant CRC cells, we next sought to determine whether this upregulation of PRMT5 in *KRAS* mutant CRC cells could be advantageously used as a treatment modality for *KRAS* mutant CRC. Our PrestoBlue™ HS Cell Viability Assay data confirmed that the upregulation of PRMT5 in *KRAS* mutant CRC was indeed clinically significant. PRMT5 inhibition resulted in a marked decrease in cell viability in CRC cells, and further displayed a substantially greater decrease in cell viability in the *KRAS* mutant CRC cells when compared to the *KRAS* WT CRC cells.

Having ascertained that inhibiting PRMT5 results in a substantial decrease in cell viability, a Guava® Annexin Red Assay was then performed to identify whether this decrease in cell viability seen previously was due in part to an increase in the number of apoptotic cells. The results of our Guava® Annexin Red Assay supported that the *KRAS* mutated CRC cells underwent a significantly greater degree of apoptosis when compared to the *KRAS* WT CRC cells.

Finally, we examined whether any additional decrease in cell viability observed in our PrestoBlue™ HS Cell Viability Assay was due in part to an increase in the number of cells undergoing cell cycle arrest. The data from our Guava® Cell Cycle Assay verified that the *KRAS* mutant CRC cells showed a greater number of cells arrested in the G2 cell cycle phase when compared to the *KRAS* WT CRC cells.

Our research strongly supports that PRMT5 is highly over-expressed in *KRAS* mutant CRC cells when compared to *KRAS* WT CRC cells. Moreover, PRMT5 inhibition in *KRAS* mutant CRC cells results in a significantly higher decrease in cell viability, and an increase in apoptosis and G2 phase cell cycle arrest, when compared to *KRAS* WT CRC cells. Our research thus suggests that a PRMT5 inhibiting treatment may prove to be effective for *KRAS* mutant CRC patients.

Further research is currently underway to determine how exactly PRMT5 and *KRAS* interact. Any information found regarding the exact molecular mechanism behind which PRMT5 and *KRAS* crosstalk can be therapeutically utilized towards developing new effective treatments for *KRAS* mutant CRC patients.

References

1. Siegel, R.L., Miller, K.D., Goding Sauer, A., Fedewa, S.A., Butterly, L.F., Anderson, J.C., Cercek, A., Smith, R.A. and Jemal, A. (2020), Colorectal cancer statistics, 2020. CA A Cancer J Clin. doi:10.3322/caac.21601
2. Jass JR. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. Histopathology. 2007;50:113–130.
3. Fearon ER. Molecular genetics of colorectal cancer. Annu Rev Pathol. 2011;6:479–507.
4. Ong, & Lee, & Tang, & Yap, Wei Hsum. (2019). Honokiol: A Review of Its Anticancer Potential and Mechanisms. Cancers. 12. 48. 10.3390/cancers12010048.
5. Cohen RB. Epidermal growth factor receptor as a therapeutic target in colorectal cancer. Clin Colorectal Cancer 2003; 2: 246251 [PMID: 12620146 DOI: 10.3816/CCC.2003.n.006]
6. Mendelsohn J, Baselga J. The EGF receptor family as targets for cancer therapy. Oncogene 2000;19:6550–65.
7. Zhao B, Wang L, Qiu H, et al. Mechanisms of resistance to anti-EGFR therapy in colorectal cancer. Oncotarget. 2017;8(3):3980-4000.
8. Van Emburgh BO, Sartore-Bianchi A, Di Nicolantonio F, Siena S, and Bardelli A. Acquired resistance to EGFR- targeted therapies in colorectal cancer. Mol Oncol. 2014; 8: 1084-94. doi: 10.1016/j.molonc.2014.05.003.
9. Brand TM, Wheeler DL. KRAS mutant colorectal tumors: past and present. Small GTPases. 2012;3(1):34-9.
10. K. Giehl Oncogenic Ras in tumour progression and metastasis Biol Chem, 386 (2005), pp. 193-205

11. Y. Pylayeva-Gupta, E. Grabocka, D. Bar-Sagi RAS oncogenes: weaving a tumorigenic web Nat Rev Cancer, 11 (2011), pp. 761-774
12. J. Downward Ras signalling and apoptosis Curr Opin Genet Dev, 8 (1998), pp. 49-54
13. Bos JL. ras oncogenes in human cancer: a review. Cancer Res. 1989; 49: 4682-9.
14. Porru, M., Pompili, L., Caruso, C. et al. Targeting KRAS in metastatic colorectal cancer: current strategies and emerging opportunities. J Exp Clin Cancer Res 37, 57 (2018). https://doi.org/10.1186/s13046-018-0719-1
15. Blanc, R.S. and S. Richard, Arginine Methylation: The Coming of Age. Mol Cell, 2017. 65(1): p. 8-24.
16. Baylin SB, Jones PA. Epigenetic Determinants of Cancer. Cold Spring Harb Perspect Biol. 2016;8(9)
17. Zhang, B., et al., Targeting protein arginine methyltransferase 5 inhibits colorectal cancer growth by decreasing arginine methylation of eIF4E and FGFR3. Oncotarget, 2015. 6(26): p. 22799-811.
18. Koh, C.M., Bezzi, M. & Guccione, E. The Where and the How of PRMT5. Curr Mol Bio Rep 1, 19–28 (2015). https://doi.org/10.1007/s40610-015-0003-5
19. Tanaka H, Hoshikawa Y, Oh-hara T, et al. PRMT5, a novel TRAIL receptor-binding protein, inhibits TRAIL-induced apoptosis via nuclear factor-kappaB activation. Mol Cancer Res. 2009;7(4):557-69.
20. Cho EC, Zheng S, Munro S, et al. Arginine methylation controls growth regulation by E2F-1. EMBO J. 2012;31(7):1785-97.

Acknowledgments

First and foremost, I would like to thank my PI and mentor Dr. Radhashree Maitra for all of her continued support and guidance throughout the past two and a half years. I would not have accomplished all that I have accomplished without all of her guidance and advice. I would also like to thank my fellow lab members who helped me with this project: Moshe Pahmer, Tzuriel Sapir, and Adam Haimowitz. I would further like to thank our laboratory technician Raymond Reynoso for all of his support and assistance throughout this project. Additionally, I would like to thank the Yeshiva College biology department for providing me with the instruments and education that were needed to reach my goals with this work. Lastly, I also would like to thank the Yeshiva University Office of the Provost for providing funding and summer stipend support for this research.