Validation of Changes in Plasma miRNA Before and After Excision of Benign and Malignant Colorectal Neoplasia

Kayla Feagins AS1, Jane Carter MB ChB2, Uri Netz MD3, M. Robert Eichenberger MS3, Susan Galandiuk MD1

1Price Institute of Surgical Research and the Section of Colorectal Surgery, Hiram C. Polk Jr. MD Department of Surgery, University of Louisville School of Medicine, Louisville, KY

Introduction

• Colorectal cancer (CRC) is common worldwide and is the second leading cause of cancer death.
• The best characterized pathway leading to the development of CRC is the adenoma-carcinoma sequence: normal colon epithelium → colorectal Advanced Adenomas (CAA) → Colorectal Adenocarcinoma.
• miRNAs (miRNAs) are short, non-coding RNAs that play an important role in gene expression.
• miRNAs have been associated with both the diagnosis and regulation of different disease processes.
• They are closely associated with cell differentiation, proliferation, and apoptosis, all very important processes in tumorigenesis.
• Current plasma-based assays used for monitoring response to therapy lack specificity and sensitivity for detecting recurrence of disease.
• We believe miRNAs have a potential role in monitoring therapy following removal of a colorectal adenoma or cancer.
• We have previously identified changes before and after excision of plasma miRNA expression in patients with CAA and CRC.
• Our aim is to validate a panel of 11 significantly dysregulated miRNAs identified from screening of plasma samples from patients obtained before and after endoscopic or surgical removal of colorectal neoplasia.

Hypothesis

• We hypothesize that miRNA expression differs between pre-treatment samples and post-removal samples of colorectal neoplasia.

Methods

• Following informed consent, blood samples were taken from patients prior to and after removal of colorectal neoplasia.
• 12 patients with advanced CAA (>0.6 cm diameter) or villous component
• 12 patients with stage II or III CRC.
• Plasma was isolated from each patient sample (n=48).
• Total RNA was extracted from plasma (Qiagen® miRNeasy) and quality and quantity were assessed.
• Reverse Transcription followed by quantitative real-time polymerase chain reaction (qRT-PCR) was performed using specific primers and probes to the 11 miRNAs of interest.
• Data analyzed using paired t-tests after normalizing raw cycle threshold data to endogenous miR-16 and RNU6.

Results

Sample Selection

Colorectal Advanced Adenoma (n=12)

<table>
<thead>
<tr>
<th>Pre-excision</th>
<th>Post-excision</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR16</td>
<td>0.913 ± 0.025</td>
</tr>
<tr>
<td>miR32-3p</td>
<td>6.032 ± 0.453</td>
</tr>
<tr>
<td>miR28c</td>
<td>3.467 ± 0.234</td>
</tr>
<tr>
<td>miR212</td>
<td>-1.957 ± 0.979</td>
</tr>
<tr>
<td>miR21</td>
<td>0.905 ± 0.524</td>
</tr>
<tr>
<td>miR22</td>
<td>-0.553 ± 1.456</td>
</tr>
<tr>
<td>miR21</td>
<td>5.007 ± 3.327</td>
</tr>
<tr>
<td>miR374a</td>
<td>3.009 ± 1.655</td>
</tr>
</tbody>
</table>

Validation of Changes

CRC

<table>
<thead>
<tr>
<th>Target Name</th>
<th>△CT Pre</th>
<th>△CT Post</th>
<th>△CT</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR16</td>
<td>0.913</td>
<td>1.664</td>
<td>0.753</td>
</tr>
<tr>
<td>miR32-3p</td>
<td>6.032</td>
<td>1.803</td>
<td>-0.032</td>
</tr>
<tr>
<td>miR28c</td>
<td>3.467</td>
<td>0.510</td>
<td>-2.957</td>
</tr>
<tr>
<td>miR212</td>
<td>-1.957</td>
<td>0.845</td>
<td>-0.112</td>
</tr>
<tr>
<td>miR21</td>
<td>0.905</td>
<td>1.347</td>
<td>-0.458</td>
</tr>
<tr>
<td>miR22</td>
<td>-0.553</td>
<td>2.539</td>
<td>3.082</td>
</tr>
<tr>
<td>miR21</td>
<td>5.007</td>
<td>2.659</td>
<td>-2.348</td>
</tr>
<tr>
<td>miR374a</td>
<td>3.009</td>
<td>0.726</td>
<td>-2.283</td>
</tr>
</tbody>
</table>

CRC

<table>
<thead>
<tr>
<th>Target Name</th>
<th>△CT Pre</th>
<th>△CT Post</th>
<th>△CT</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR16</td>
<td>0.913</td>
<td>1.664</td>
<td>0.753</td>
</tr>
<tr>
<td>miR32-3p</td>
<td>6.032</td>
<td>1.803</td>
<td>-0.032</td>
</tr>
<tr>
<td>miR28c</td>
<td>3.467</td>
<td>0.510</td>
<td>-2.957</td>
</tr>
<tr>
<td>miR212</td>
<td>-1.957</td>
<td>0.845</td>
<td>-0.112</td>
</tr>
<tr>
<td>miR21</td>
<td>0.905</td>
<td>1.347</td>
<td>-0.458</td>
</tr>
<tr>
<td>miR22</td>
<td>-0.553</td>
<td>2.539</td>
<td>3.082</td>
</tr>
<tr>
<td>miR21</td>
<td>5.007</td>
<td>2.659</td>
<td>-2.348</td>
</tr>
<tr>
<td>miR374a</td>
<td>3.009</td>
<td>0.726</td>
<td>-2.283</td>
</tr>
</tbody>
</table>

Conclusions and Future Directions

• We validated expression levels of 5 miRNAs (miR-32-3p, miR-454, miR-29c, miR-372 & miR-374a) to be different in pre-treatment compared to post-removal plasma samples in patients with CRC and CAA.
• miR-374a was significantly downregulated in both CRC and CAA in pre-treatment samples compared to post-treatment samples.
• These findings may help provide for a relatively non-invasive method of monitoring therapy or assessing response to treatment, and can be used as an innovative tool in diagnostics.
• Future considerations should include more specific miRNAs that play a role in initiation, progression, and outcomes in colorectal cancer and the use of miRNAs as potential therapeutic targets.

Acknowledgments

National Cancer Institute grant R25-CA134283, John Williamson and Barbara Thurston Atwood Price Trust
Rest-activity Rhythms and Quality of Life in Lung Cancer Patients

Oliveia Fields1, Elizabeth Cash, Ph.D.1,2,3, Lauren A. Zimmaro, MA1, Allison Hicks, BA1, Christy Albert, BS2, Sandra E. Sephton, Ph.D.1,2

1Department of Psychological and Brain Sciences, University of Louisville, Louisville, KY.
2J.G. Brown Cancer Center, University of Louisville, Louisville, KY.
3Department of Otolaryngology- HNS and Communicative Disorders, University of Louisville School of Medicine, Louisville, KY.

Learning Objective

This study aims to examine the relationship between rest-activity rhythms and quality of life in lung cancer patients as both are strong predictors in cancer survival and prognosis.

Background

Lung cancer is the second most common cancer in men and women. Distress from initial diagnosis and continued lung cancer treatments can lead to a decline in quality of life (QOL). Numerous studies have shown quality of life to be a significant prognostic and survival predictor in lung cancer patients1. Quality of life is a multidimensional concept that includes subjective evaluations of positive and negative aspects of life. A reliable QOL survey pertaining to lung cancer patients is the Functional Assessment of Cancer Therapy-Lung Cancer (FACT-L), which includes items and scales shown in Figure 1. Circadian coordination has been shown to have strong tumor-suppressive actions in laboratory and animal studies2. In humans, circadian rhythms of rest-activity cycles are prognostic for colorectal cancer survival and may predict response to treatment3. Circadian rhythms are a system composed of molecular clocks that synchronize physiological functions, such as the cell cycle and apoptosis, in a 24-hour cycle4. Rest-activity rhythms are also one of the many circadian rhythms in the body. There is evidence that disruption of circadian rhythms can lead to carcinogenesis5,6. There is a scarcity of research on the relationship between these two prognostic factors in lung cancer patients: rest-activity rhythms and quality of life. Therefore, this study aims to evaluate the relationship between these two variables among a sample of lung cancer patients.

Hypothesis

Lung cancer patients with more disruptive rest-activity rhythms will have lower quality of life and poorer scores on FACT-L.

Methods

Participants

Non-small cell lung cancer patients (N=49; 14 males, 35 females) diagnosed within the last 5 years provided demographics and self-report measures of quality of life (FACT-L). Participants wore an actigraphy watch for 10 consecutive days and filled out daily questionnaires giving time of wake and bed along with times the watch was removed.

Measures

To calculate rest-activity rhythms (ACT) graph Actionkit software was used, and data were binned over time in bed (TBI) intervals. Intervals were marked based on the daily questionnaire and visual inspection of the data. The software calculated autocorrelation coefficient (r24), a measure of regulation of daily activity calculated by taking a correlation of 1-minute epochs on one day with the same epoch on different days to give a 24-hour correlation; nighttime restlessness (r01), the percent of time in bed in which activity falls below the median of activity out of bed (higher scores indicate more restlessness); and disharmony (Dh), the percent of time out of bed in which activity falls below the median of activity out of bed (higher scores indicate more sedentary behavior).

The Functional Assessment of Cancer Therapy-Lung Cancer (FACT-L) is a 44-item self-report instrument, which measures multidimensional quality of life of lung cancer patients. Measures are listed in Figure 1.

Statistical Analysis

Prior to analysis, all data were transformed into z-scores. Separate regressions were run using the independent and dependent variables for each pathway outlined in Figure 1, resulting in a total of 18 regressions. In all hierarchical multiple regressions, control variables (gender, age of diagnosis, cancer stage, income) were entered in Model 1. In Model 2, independent were added to the overall models predicting overall health-related quality of life scores, and the subscale scores.

Results

Hierarchical regressions revealed significant relationships between nighttime restlessness and physical well-being (PWB). Lung cancer patients with more nighttime restlessness had higher PWB scores (p= .016). Age was also a significant predictor such that younger patients had poorer well-being when r01 was the predictor and PWB was the outcome (p= .043). Significant results were also present when controlling for the following relationships between I/O and social-family well-being (SFWB) (p=.026), and I/O and SFWB (p=.028), indicating older lung cancer patients have higher social-family well-being scores. No significant relationships were found in this study.

Conclusions

At present, nighttime restlessness and PWB, in addition to being related to cancer prognosis and survival rates in other cancers, are significantly related to each other in lung cancer patients.

Poor physical well-being in younger patients may reflect more aggressive tumors, although stage was not associated with QOL, suggesting QOL gives different information than stage for prognostics and course of disease.

Older lung cancer patients had higher social-family well-being. This could be due to more developed long-term relationships in older individuals, in general.

Several other studies have suggested QOL and rest-activity rhythms provide useful information for cancer prognosis and survival3,5,7. Many have also suggested these two variables should be considered in cancer survival diagnosis in multiple cancer6. This study supports both these notions as understanding their relationship could give more information on their prognostic value and support their integration in diagnosis.

Future research should continue to explore the relationship between rest-activity rhythms and quality of life.

Further investigation of the association between rest-activity rhythms and QOL could reveal a third driving variable, such as endocrine or immune function.

Acknowledgements

Funding support was provided by National Cancer Institute Grant CA21442 and CA167104 (S. E. S.).

References

In conclusion, to play cells, Mesenchymal significantly and Cancer DE that are significantly downregulated in the presence

455.361 it DE 5.640644 considered 26.4322 tested 4.892256 designed in 2013 Feb;92(2):114 have not been investigated in OSCC, and have are RNAs that are >200bp ANOVA expression may help identify novel regulatory

gingivalis to 40.8831 effect in increased invasiveness and cell P is an oral NIDCR RT gingivalis were were designed in our lab from gingival epithelial cells and have been shown when infected with PG to undergo EMT. There are limited biomarkers to identify oral squamous cell carcinoma and its progression, so investigating IncRNA expression may help identify novel biomarkers.

Methods: Telemereas immortalized gingival keratinocytes were grown to 60-80% confluence, and then infected with P. gingivalis. RNAseq data for non infected and P. gingivalis infected TIGK cells were aligned to human reference genome Hg37 using TopHat, and differential expression of IncRNAs between no infection (NI) and infected TIGK cells were analyzed using Cufflinks. P-values ≤ 0.05 were considered significant.

Results: Rinc00152 showed significant upregulation by P. gingivalis in infected TIGK-cells compared to the control no infected TIGK-cells.

Conclusions: The IncRNA investigated in this study was significantly regulated by P. gingivalis. P. gingivalis can induce epithelial to mesenchymal transition in TIGK cells, which is a biomarker for oral squamous cell carcinoma. The purpose of this regulation is not yet understood, but investigating the mechanism by which these IncRNAs operate within the cell will be useful in identifying oral squamous cell carcinoma in patients presenting with OSCC symptoms.

48-250 Americans are diagnosed with Oral Cancer each year, and the survival rate at 5 years is approximately 57% according to the oral cancer foundation. Oral Squamous Cell Carcinoma appears asymptomatic at first, and has limited diagnostic biomarkers to identify the progression of the tumor. Epithelial to Mesenchymal Transition (EMT) markers are used to identify the cancer currently. EMT is the process by which the normal epithelial tissue has undergone a morphological change, and adopts an invasive myofibroblast phenotype as seen in Figure 2. The now mesenchymal cells can invade the basement membrane, enter the bloodstream, and then undergo Mesenchymal to Epithelial transition to aggregate and metastasize. EMT is an oral gram negative pathogen that has been implicated in OSCC. One of the mechanisms by which EMT has been characterized in gingival epithelial cells is the upregulation of ZEB1, which is a key transcriptional regulator in the transition to this invasive phenotype. Long non-coding RNAs are RNAs longer than 200bp, and lack protein coding potential. Recent studies have implicated IncRNAs in increased invasiveness and cell proliferation in gastric, liver, and kidney cancer[9]. IncRNAs have not been investigated in OSCC, and have been shown to be significantly upregulated regulated based on preliminary RNAseq statistical analysis of telemereas immortalized keratinocytes infected with P. gingivalis.

References

Characterization of acidic pH functionalized mesoporous silica nanoparticles for ovarian cancer diagnostics

Benjamin Fouts, Phillip Chuong, Lacey R. McNally

Department of Medicine, University of Louisville, Louisville, KY

ABSTRACT

Purpose: Current FDA approved clinical screening approaches for ovarian cancer, such as CA-125 and TVUS, have been shown in recent years to provide minimal improvements to overall survival rates. This is due to the majority of ovarian cancer diagnoses coming at later stages of the disease, where detection could have aided patients in pursuing otherwise more effective treatment options. New diagnostic tools need to be developed to help detect ovarian cancer at an earlier stage such as by increasing detection in liquid biopsies.

Methods: We utilized a modified Stober method to generate small, mesoporous silica nanoparticles (MSNs) based upon tetraethylorthosilicate (Scheme 1). Following coating in acetic acid and ammonium nitrate (ANI), the core MSNs were then conjugated with (3-aminopropyl)triethoxysilane (APTES) and 3-(2-chloroethyl)-3-(2-chloroethyl)aminopropyltrimethoxysilane (GTECS) before being coated with a polyanion solution. At a low pH, MSN-80 dye was introduced and encapsulated. The MSN core further conjugated with 4-(2-Maleimidomethyl)phenylsulfonic acid and 6-hydrazinocarbonylazine eser (SMC) and ε-PAH to produce a biologically active dye. This dye, blc labeled, was conjugated with AMI (CVM-Me) for further imaging in vivo.

Results: CVM-Me visualization was observed in ovarian cancer cell lines and in vivo. A summary of the characteristics of CVM-Me’s size, average particle size of 25.8 ± 3.3 nm was calculated by measuring 20 images on Image J software.

Conclusions: We synthesized an MSN of 25.8 ± 3.1 nm., increasing M-Me core size significantly for M-Me core particles, not for P-Pf. This is due to the size of the nanoparticles that are being used.

Acknowledgements:

We would like to thank all the staff and faculty at the MIRC for their help, through the KSG-C-L ICULAB grant, especially for support of the course of liquid biopsy analysis at the School of Medicine. Further, we are thankful for the efforts of Dr. Mitchell, throughout this experimentation.
Invasion Assay

GFP, Cas9, and either guide RNA 2 or 5. Cells were sorted/collection for GFP expression and CRISPR/Cas9 Constructed NAT1 Knockout

Methods

Hypothesis

the ability of those cells to grow in close proximity to one another. of NAT1 in MDA-MB-231 cells did not affect anchorage-independence or invasion, but rather anchorage-independent growth and the cellular invasion ability (P= 0.19). Thus, the absence

anchorage-dependent apoptosis (anoikis) and have a decreased invasive ability compared to the parental line. Both the CRISPR/Cas9-mediated NAT1 knockout and the parental line were compared side by side in anoikis assays spanning six days. Transwell® invasion, and migration assays. Albeit prior literature reported a decrease in invasive ability in vitro, we determined that the absence of NAT1 in MDA-MB-231 cell lines had little to no effect on anchorage-independent growth and the cellular invasion ability (P= 0.19). Thus, the absence of NAT1 in MDA-MB-231 cells did not affect anchorages-independent or invasion, but rather the ability of those cells to grow in close proximity to one another.

Results

As supported by previous literature, we hypothesize that the CRISPR/Cas9-mediated NAT1 knockouts will have a decreased invasive ability in MDA-MB-231 cell lines compared to the parental line. The dramatic differences in soft agar colony formation led us to hypothesize that the CRISPR-Cas9-mediated knockouts may have a decreased ability for anchorage-independent growth.

Results

Figure 1 – Characterization graphs of MDA-MB-231 parental and CRISPR/Cas9 NAT1 knockout cell lines. A) The in vitro NAT1 enzymatic activity. B) The in vitro NAT1 activity of the cells. There was no detectable activity in either measure of activity (A or B) for the knockout cell lines. C) The doubling time in hours for the three cell lines. There was no difference between the three cell lines. D) The number of colonies able to form in a soft agar assay. There was a significant reduction of soft agar colony formation for the CRISPR/Cas9 cell lines compared to the parental line.

Figure 3 – Comparison of photomicrographs taken of the bottom side of Transwell® insert membranes. This figure illustrates the different number of cells that were able to reach the bottom side of the membrane, either with or without gel for parental or CRISPR/NAT1 knockout cell lines. The gel concentration is given along the top of the columns as mg/ml and then directly below is the number of cells that were seeded into the Transwell® insert.

Figure 4 – Relative Invasion ability for MDA-MB-231 parental and NAT1 knockout cell lines. Relative invasiveness was calculated by dividing the cells that invaded through the 0.5 mg/ml gel matrix (insert seeded with 50,000 cells) by the number of cells that migrated through no gel (insert seeded with 25,000 cells). The resulting one-way ANOVA of the relative invasiveness value for each cell line was non-significant (P=0.19; N=4).

Conclusions

We examined anoikis resistance and invasive ability of NAT1 knockouts in MDA-MB-231 breast cancer cell lines. Previous data from our lab showed the CRISPR/Cas9-mediated NAT1 knockouts yielded significantly fewer colonies in soft agar assays. To further understand what may be causing the difference soft agar assays, we investigated the CRISPR/Cas9 NAT1 knockouts resistance in comparison to their parental lines. The data showed that NAT1 knockout cell lines retained the same level of anoikis resistance as the parental cell line. In this study we also investigated NAT1 knockout cell lines ability to invade through the Transwell® membrane. The results suggest that there was no significant difference between parental and NAT1 knockout cell lines. Further studies may examine the mechanism by which MDA-MB-231 NAT1 knockout cell lines ability to inhibit their own growth in close proximity. Understanding NAT1’s role in breast cancer may lead to a novel approach in inhibiting cellular metastasis.

Acknowledgements

Research funding was supported by the University of Louisville and the National Cancer Institute R25-CA134283 grant.

A special thanks to the Hein lab and members from States, Kidd, Siskind, and Beverly labs.
INTRODUCTION

Mutationally activated RIT (RIT-Q79), a Ras-related GTP binding oncoprotein, was not considered important in carcinogenesis until recently. RIT is now identified as a key oncoprogenic mutation in about 3% of lung cancer cases, determined by the mass genomic sequencing of tumors. Lung cancer is the most common cause of cancer death in the United States for both men and women. Every year, this translates to more than 5,000 people being diagnosed with incurable RIT-driven lung cancer. Yet, for the process by which mutationaly activated RIT contributes to lung cancer as well as the signal transduction pathways involved remain largely unknown. We have identified the tumor suppressor NORE1A as a novel direct binding partner of RIT in a yeast two-hybrid screen. Here we show that NORE1A and RIT can complex in mammalian cells and that NORE1A acts as a protection mechanism against RIT-mediated hyper-stimulation of growth and development pathways. Suppression of NORE1A expression was essential for RIT to express its transforming phenotype. In tumor cells, NORE1A is often silenced by epigenetic inactivation hence, knockdown of its expression will facilitate tumor formation due to RIT. The importance of identifying and characterizing this interaction, as well as understanding its mechanism of action in the event of lung carcinoma may lead to the development of better targeted therapies for RIT-driven lung cancer.

METHODS

Cell Lines: NIH-3T3 cells are a mouse fibroblast cell line and were maintained in DMEM with 10% calf serum (CS) and 1% penicillin-streptomycin and HEK293 cells in DMEM with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. NIH-3T3 and HEK293 is stably transfected with a scrambled control shRNA, NIH-3T3-shSCRAM and NIH-3T3-shNORE1A 3' and NIH-3T3-shNORE1A 971 were knocking down for NORE1A expression. All three cell lines NIH-3T3-shSCRAM, NIH-3T3-shNORE1A 3' and NIH-3T3-shNORE1A 971 were constructed and generously gifted by Dr. Howard Donninger.

Luciferase Assays: HEK293 cells are human epithelial cells that were transfected with expression constructs tagged with fluorescent protein (GFP) or human influenza hemagglutinin (HA), incubated for 24 hours and then lysed. NIH-3T3 cells were transfected with expression constructs tagged with fluorescent protein (GFP) or human influenza hemagglutinin (HA), incubated for 24 hours and then lysed. Gene expression in HEK293 and NIH-3T3 cells was measured using a Dual Luciferase Kit from Promega along with a Berthold Technologies Lumat LB9507 tube luminometer.

Proliferation Assays: NIH-3T3 cells were seeded in the wells of a 6 well plate. Each cell line was transfected with expression constructs tagged with fluorescent protein (GFP) and incubated at 37°C. Cell counts were taken every 24 hours using a hemacytometer.

Soft Agar Colony Formation Assays: NIH-3T3 cells were transfected with expression constructs tagged with fluorescent protein (GFP), suspended in 0.35% agar solution and seeded on 0.7% agar base in the wells of a 6 well plate. The cells were incubated at 37°C for two weeks before colony counts were measured.

RESULTS

Figure 1: NORE1A interacts with the smal-GTPase RIT. NORE1A and RIT were co-expressed identified as binding partners by a yeast two-hybrid screen. We sought to confirm this result by using two different assays. First, fluorescence microscopy images of GFP-RIT plated with a RIT-KATE tagged NORE1A shows that these two proteins co-localize on both the cellular membrane as well as in punctate structures in the nucleus, which is consistent with an established co- fusion formation where cells transfected with GFP-tagged wild-type RIT and mutationaly activated RIT, were immunoprecipitated using GFP agarose beads. Here we found that NORE1A binds strongly to both wild-type and mutationaly activated RIT.

Figure 2: NORE1A suppresses RIT mitogenic signaling. HEK293 cells were transfected with GFP-vector, GFP-tagged RIT Q79 or GFP-tagged RIT Q79 and HA-tagged NORE1A. When RIT is mutationaly activated, large increases are observed in both Gαq (p<0.05) and NFκB transcriptional activity. When NORE1A is co-expressed with activated RIT, Gαq and NFκB activation are suppressed in HEK293 cells. NIH-3T3 cells transfected with GFP-RIT via a lentiviral vector were transfected with double and a lentiviral vector control containing with previously validated shRNA constructs for NORE1A including a scrambled vector control and two different shRNA constructs. Suppression of NORE1A allows activated RIT to more powerfully activate Gαq and NFκB transcriptional activity (p<0.05) supporting our results from the HEK293 cells, in regards to NFκB activity in NIH-3T3 cells with mutationaly activated RIT, we did not observe NORE1A following a similar trend.

Figure 3: Schematic of proposed RIT Q79/NORE1A tumor suppressor pathway. NORE1A binds and suppresses mutationaly activated RIT oncoprotein directly, resulting in the suppression of RIT growth and transformation signaling via Gαq pathway regulation and NFκB growth and transformation signaling control. In human cancers, loss of NORE1A expression prohibits this functional interaction, leading to excessive proliferation signaling through a number of pathways including Gαq/11 and NFκB signaling mechanisms.

DISCUSSION

RIT has been now identified as a driving mutation in about 3% of lung cancer cases, although the signal transduction process by which mutationaly activated RIT may contribute to cancer remains unknown. We have identified a tumor suppressor called NORE1A as a direct binding partner of RIT. Studies in which we examined the action of RIT in cells knocked-down for NORE1A showed that NORE1A suppresses both the mitogenic signaling and the transforming activity of RIT. As NORE1A is frequently inactivated by promoter methylation in lung tumors, NORE1A inactivation may be essential for the development of RIT driven lung cancer.

Conclusions

1. NORE1A and RIT form a complex in mammalian cells.
2. NORE1A suppresses the mitogenic and tumorigenic phenotype of RIT oncogores.
3. Epigenetic therapy to restore NORE1A expression may be a novel approach to suppress RIT-driven lung cancer.

ACKNOWLEDGEMENTS

Research was supported by the NIH R25 Grant University of Louisville Cancer Education Program (1R25-CA134283). Special thanks to the Dr. Lee Schmidt and Clark lab for their assistance with this project.

Functional Interaction Between NORE1A and Mutationaly Activated RIT(Q79)
Represses Malignant Transformation in Lung Cancer
Ashley M. Gleaton¹, M. Lee Schmidt, Ph.D. ¹,², and Geoffrey J. Clark, Ph.D.¹,²
¹ Molecular Targets Group, James Graham Brown Cancer Center and ² Department of Pharmacology & Toxicology
School of Medicine, University of Louisville, Louisville, KY, USA
Chronic medication use in chronic diseases along with chronic cancer treatment could lead to further medical conditions as a result of inadequate monitoring. The percent of older adults is growing and as such, the percent of persons living with breast cancer as a chronic disease is growing. (1) One medication which is a corner stone of long term breast cancer therapy is Tamoxifen.

The traditional course of treatment spans approximately 5 years, and since the findings of the ATLAS study in 2015, the standard of care will increase the average length of Tamoxifen treatment to 10 years. (2)

Adverse drug events account for a high percentage of hospitalizations in older adults and estimates reveal that up to 33% of all hospitalizations in older adults are due to medication related problems (3) (One means of preventing adverse drug events is through adequate monitoring and pharmacovigilance. (4,5)

The medical literature describes the common adverse events which with tamoxifen is associated. (6) Namely, hypercalcemia (especially with initiation of therapy and in patients with bone metastasis), deep vein thrombosis (DVT), pulmonary embolism (PE), stroke, and uterine malignancy.

Appropriate pharmacovigilance for chronic Tamoxifen use also includes: tolerability, duration of therapy, drug interactions, and drug disease interactions (contraindications).

### Hypothesis

We hypothesize that individuals being administered Tamoxifen are not being appropriately monitored which could lead them to increased risk of medical conditions arising such as hypercalcemia, uterine malignancies, or stroke.

### Methods

We requested identification of patients who are currently taking tamoxifen regardless of age, or practice, from all of the outpatient clinics within our healthcare system. The confidential and HIPPA protected data provided by our health system IT data specialist yielded 93 patients receiving tamoxifen therapy. A patient identification key was created, numbering each patient sequentially and the key kept separated and securely from the patient identification list.

The data collected includes the electronic health record of: indication for tamoxifen use, drug allergies, tamoxifen dose, duration of tamoxifen therapy, presence of calcium supplementation, concurrent use of bone density medications, presence and date of calcium level monitoring, PTH level, documentation of deep vein thrombosis, pulmonary embolism or stroke monitoring, presence of a history of hypercoagulability (DVT, PE, or stroke), bone metastasis, monitoring for uterine malignancies, history of uterine malignancies, warfarin or anticoagulation use. Our data focuses on the presence or lack of documentation regarding said tamoxifen use.

### Results

<table>
<thead>
<tr>
<th>Data Collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Charts/Subjects</td>
</tr>
<tr>
<td>Age Range of Subjects</td>
</tr>
<tr>
<td>Average Age of Tamoxifen User</td>
</tr>
<tr>
<td>Missing Indication for Tamoxifen Use</td>
</tr>
<tr>
<td>Duration of Therapy Documentation (ATLAS study: 10 yrs.)</td>
</tr>
<tr>
<td>Calcium Monitoring Absent</td>
</tr>
<tr>
<td>No Calcium Level Documented Within Past Year of Levels on Record</td>
</tr>
<tr>
<td>Absent documentation of screen for DVT, PE or Stroke (Increased clot risk with Tamoxifen use.)</td>
</tr>
<tr>
<td>Absent documentation of monitoring for uterine malignancies.</td>
</tr>
<tr>
<td>Patients receiving Warfarin or other Anticoagulant therapy. (Tamoxifen is pro-thrombic)</td>
</tr>
<tr>
<td>Patients receiving concurrent Warfarin or other Anticoagulant therapy.</td>
</tr>
</tbody>
</table>

We hypothesized that individuals being administered Tamoxifen are not being appropriately monitored which could lead them to increased risk of medical conditions arising such as hypercalcemia, uterine malignancies, or stroke.

### Discussion

We hypothesized that individuals being administered Tamoxifen are not being appropriately monitored which could lead them to increased risk of medical conditions arising such as hypercalcemia, uterine malignancies, or stroke. Our results reveals consistent gaps in monitoring, despite the rare case of attempts to provide appropriate monitoring.

As the clinical adage goes: “If it is not documented, it didn’t happen”. A significant source of lack of documentation appears to be incomplete records. For example, a patient seen by our system for reconstructive surgery, where the sole documentations are the medications and surgery procedure summary note for clinic follow up, devoid of any laboratory findings or progress notes. This gap in documentation causes a missed opportunity for each clinician who accesses the patient chart to have the data at hand to perform pharmacovigilance and drug use surveillance. Most serious adverse drug events are not discovered until the case enters the ER door, and 1 in 3 hospital admissions in older adults are due to adverse drug events, yet, appropriate drug use monitoring is one means to prevent unnecessary medication related harm. (7,8)

The average office visit consists of 15 minutes, of which 7 are used for establishment of the topic or problem to work on, leaving 3.5 for problem solving and 3.5 for medication management. (9) Thus, it is easy to understand why patient records are incomplete. The mandate to use EHRs by the affordable care act was a step towards the possibility of universal health records, which may some day help improve the problem of incomplete charts.

### Conclusions

This study provides a description of the current state of Tamoxifen Pharmacovigilance and can be a starting place for benchmarking improvements in Tamoxifen safety and use quality improvement interventions.

### Acknowledgments

National cancer Institute R25 research Grant and the James Brown Cancer Center Summer Research Program

### References