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Research Article

Myosin Regulatory Light Chain Silencing: Function, Mechanisms, and Therapeutic Implications in Pancreatic Cancer

Jameka Grigsby[1]*, Shavonda Jackson[1], Kenneth Duma[2], Ariane M Chitoh[3] and Clement G Yedjou[4]

[1]Department of Biological Sciences, Alcorn State University, Lorman, MS, 39096, USA

[2]Department of Biology, Jackson State University, Jackson, MS, 39217, USA

[3]RCMI Center for Health Disparities Research, Jackson State University, Jackson, MS, 39217, USA

[4]Department of Biological Data Sciences, Florida Agricultural and Mechanical University, Tallahassee, FL 32307, USA

***Corresponding Author:** Jameka Grigsby, Department of Biological Sciences, Alcorn State University, Lorman, MS, 39096, USA.

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Abstract

Pancreatic cancer is the most lethal of all cancers. Patients diagnosed with this disease die within six months 95% of the time. There is one currently known conventional chemotherapy for pancreatic cancer and it provides minimal benefits. This study's recent strategic efforts have focused on identifying and validating improved molecular targets against pancreatic cancer. In this study, the potential role of Myosin Regulatory Light Chain (MRCL3) in pancreatic cancer was evaluated. MRCL3 is a regulatory protein implicated in cytokinesis, receptor capping, and cell locomotion. The effect of silencing MRCL3 in BxPC3 cells was examined to determine whether endogenous MRCL3 promoted growth in pancreatic cancer cells. This study demonstrated that MRCL3 significantly inhibited the proliferation of these cells compared with the controls. Cells undergoing apoptosis (Sub G1) were measured after transfecting BxPC3 cells with MRCL3 siRNA to determine whether MRCL3 is also required for protecting pancreatic cancer cells from cell death. Knockdown of MRCL3 resulted in 10% increase in cell death in BxPC3 cells compared to the controls. The expression of AP-1 protein factors, c-Fos and c-Jun, were examined in BxPC3 cells to investigate how MRCL3 regulated cell fate. This study has shown that MRCL3 knockdown in these cells increased the expression of c-Jun while blocking c-Fos expression. These results imply that the MRCL3 pathway is mediated through AP-1 transcriptional factors. Given the lack of promising pancreatic cancer drugs, this study sheds several insights on possible targets that can lead to the treatment of this disease.

Keywords: Myosin Regulatory Light Chain; Pancreatic Cancer; Proliferation (MTS) Assay; Western Blot; Phospho p53 Assay

Abbreviations

ADP: Adenosine Diphosphate; Arg-13: Arginine at Position 13; Asn→Lys: Asparagine to Lysine Region; ASPC-1: Type of Pancreatic Cell Line (Adenocarinoma); ATP: Adenosine Triphosphate; ATPase: Adenosine Triphosphate Enzyme; A13T: Alanine to Threonine Myosin Regulatory Light Chain Mutant; A549: Type of Lung Cancer Cell Line (Carcinoma); BDM: 2, 3-Butanedione Monoxime; BxPC3: Biopsy Xenograft of Pancreatic Carcinoma Line 3; CAFs: Cancer-Associated Fibroblasts; Capanc-1: Type of Pancreatic Cancer Cell Line (Carcinoma); Casp 3: Caspase 3; CEFs: Chick Embryo Fibroblasts; c-Fos: Protein Encoded by Fos Gene; c-Jun: Product of c-Jun Gene; CMF: Cardiac Myofibrils; CO2: Carbon Dioxide; CTnC: Cardiac Troponin C; D166V: Aspartic to Valine; ECL: Enhanced Chemiluminescence; E22K: Glutamic Acid to Lysine Myosin Regulatory Light Chain Mutant; FBS: Fetal Bovine Serum; FHC: Familial Hypertrophic Cardiomyopathy Phenylalanine to Leucine Myosin Regulatory Light Chain; F18L: Mutant; HCRLC: Human Cardiac Myosin Regulatory Light Chain; HEK-293: Human Embryonic Kidney; HeLa: Henrietta Lacks; HPDE: High-Density Polyethylene; H2B-GFP: Histone Fusion Protein; LC20: Myosin Regulatory Light Chain; L-jump: Length Jump; MHC: Miapaca MLC-GFP MLCK: Major Histocompatibility Complex Type of Pancreatic Cancer Cell Line (Carcinoma) Myosin Regulatory Light Chain Fusion Protein Myosin Light Chain Kinase; MPS: Multipolar Spindle Formation; MRCL: Myosin Regulatory Light Chains; MRCL2: Myosin Regulatory Light Chain 2; MRCL3: Myosin Regulatory Light Chain 3; MTS: 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide; MYPT1: Myosin Targeting Subunit; MYP2: Myopia-2 Locus; NTg: Non-Transgenic; N47K: Familial Hypertrophic Cardiomyopathy Mutation; OKF-Htert: Human Oral Keratinocyte Immortalized with Human Telomerase; OSCC: Oral Squamous Cell Carcinoma; PBS: Phosphate Buffered Saline; pCa: Force- Calcium Relationship; PI: Propidium Iodide; P1: Phase 1; P2: Phase 2; p53: Tunor Protein 53; P95A: Proline to Alanine Myosin Regulatory Light Chain Mutant; RLC: Regulatory Light Chain; ROCK1: Rho-Associated, Coiled- Coil Containing Protein Kinase 1; RPEhTERT: Human Telomerase; RPM: Revolution Per Minute; R58Q: Arginine to Glutamine Myosin Regulatory Light Chain Mutant; SCC103: Type of Oral Cancer Cell Line (Carcinoma); SDS: Sodium Dodecylsulfate; Ser-19: Serine at Position 19; SiMYPT1: Small Interfering Myosin-2 Locus; SK-HEP1: Type of Liver Cancer Cell Line (Adenocarcinoma) smRLC Small Regulatory Light Chain; S1: Myosin Crossbridges; Tg-D166V: Transgenic Aspartic to Valine; Tg-WT: Transgenic Wild Type; Tnl: Troponin l; UPCI: Type of Oral Cancer Cell Line (Carcinoma); UTR: Untranslated Region of mRNA; 5' UTR: 5' End to Position of First Codon Used in Translation of mRNA U20S Type of Osteosarcoma Cell Line.

Introduction

Myosin Regulatory Light Chain Composition

Myosin is a highly conserved, ubiquitously expressed protein that interacts with actin to generate the force for cellular movements. Conventional myosins are hexameric proteins consisting of two heavy chain subunits, a pair of nonphosphorylatable light chain subunits and a pair of phosphorylatable light chain subunits. Myosin regulatory light chain (MRCL3) has a molecular weight of 20 kDa.

Myosin regulatory light chain (MRCL3) regulates contraction in smooth muscle and non-muscle cells via phosphorylation by myosin light chain kinase (MLCK) implicated in cytokinesis, receptor capping, and cell locomotion as proven by [1]. Phosphorylation of myosin regulatory light chain is catalyzed by MLCK in the presence of calcium and calmodulin and increases the actin-activated myosin ATPase activity, thereby regulating the contractile activity. Myosin light chain is also located in striated skeletal muscle, where its function remains undefined. In a 1987 study done by [2], myosin tested using pancreatic cancer cell lines proved that pancreatic acinar cells contain a typical non- muscle myosin, and the subunits of this molecule are subject to post-translational modification by phosphorylation. This was concluded from the results that revealed pancreatic myosin bound to actin could be dissociated by the addition of Magnesium (Mg) ATP and myosin purified was phosphorylated on one of the light chains as well as the heavy chain. The ground breaking results from this study opened a whole new door for myosin and its correlation in pancreatic cancer. There are few studies examining the role of myosin regulatory light chain 3 (MRCL3) in pancreatic cancer.

Myosin regulatory light chain in cancer

In breast cancer, MRCL3 is a potential tumor suppressor gene candidate. It is known to be one of the fourteen (14) genes of gene expression that was down-regulated in relation with their copy number losses in the 2010 study of [4]. Copy number transitions are likely to reflect DNA-strand breakage that may lead to nonreciprocal translocations.

With the loss of the 18p11.31 region in the chromosome arm of the mammary gland, MRCL3 is progressively down-regulated. The deletion of the tumor suppressor gene is reported to aid in the down-regulation of the development and progression of breast cancer.

In prostate cancer, MRCL3 is one of the three genes that are present as upregulated and downregulated in cancer-associated fibroblasts (CAFs). This is suggested to be due to tag splice variants. MRCL3 can be either as a cancer-associated fibroblasts-enriched transcript or cancer-associated fibroblasts-depleted transcript in the 2011 study done by [3]. The statically analysis performed by [3] showed expression in both factors

cancerassociated fibroblasts-enriched and cancer-associated fibroblasts-depleted.

Cancerassociated fibroblasts-enriched transcripts are associated with prostate morphogenesis and cancer-associated fibroblasts-depleted are associated with cell cycle. There are few studies examining the role of myosin regulatory light chain 3 (MRCL3) in cancer. This study is interested to determine the role of myosin regulatory light chain 3 (MRCL3) in pancreatic cancer.

Pancreatic cancer

Pancreatic cancer, also termed as pancreatic carcinoma, is known to be the fourth leading cause of cancer death overall. It affects over 43,140 people per year. Pancreatic cancer is a cancer of the pancreas caused by malignant carcinoma cells originating within the tissues of the pancreas that produce hormones. The pancreas is a large organ located behind the stomach that makes and releases enzymes into the intestines that help the body absorb foods. Hormones called insulin and glucagon, which help the body control blood sugar levels, are made in special cells in the pancreas called islet cells. Tumors can also occur in these cells. The exact cause of pancreatic cancer is unknown. Many symptoms are mediated by pancreatic cancer in individuals but they are usually recognized at later stages. By this time, the cancer is already advanced when first identified. Some symptoms include pain or discomfort in the upper part of the abdomen, loss of appetite and weight loss, jaundice (a yellow color in the skin, mucus membranes, or the eyes), dark urine,

claycolored stools, fatigue, weakness, nausea and vomiting.

Rationale

It has been previously demonstrated by [1] that myosin regulatory light chain interacts with actin to generate the force for cellular movements and is implicated in cytokinesis, receptor capping, and cell locomotive. Myosin regulatory light chain in breast cancer has been shown to be a potential tumor suppressor gene candidate [4]. The deletion of the tumor suppressor gene is reported to aid in the progression of breast cancer. Since there are very little studies on myosin regulatory light chain's effect in pancreatic cancer, this study examines its role in the development of pancreatic cancer.

Hypothesis

Myosin Regulatory Light Chain is involved in mediated transcriptional factors, cell proliferation and apoptosis.

Objective

In this study, the role of myosin regulatory light chain 3 (MRCL3) in pancreatic cancer was determined.

Materials and Methods

Cell lines and reagents

Pancreatic cancer cell line, BxPC3, was obtained from the American Type Culture Collection (Rockville, MD). The BxPC3 cells were cultured in essential RPMI 1640 medium (Sigma), supplemented with 10% fetal bovine serum (FBS) (Atlanta Biologicals), 2mM L-Glutamine (Sigma), and 100 U penicillin/0.1 mg/ml streptomycin (Invitrogen Corp., Carlsbad, CA). All cells were incubated at 37°C in a humidified atmosphere containing 5% CO2 with medium changed every two days for two weeks. c-Fos antibody (1:1000; Santa Cruz Biotech), c-Jun antibody (1:1000; Santa Cruz Biotech) and Caspase 3 antibody (1:1000; Santa Cruz Biotech) were used as primary antibodies for western blot analysis.

Horseradish peroxidase-conjugated anti-mouse antibody whole IgG (1: 5000; Santa Cruz Biotech) were used as a secondary antibody for all three primary antibodies.

Transfections

For transfection of BxPC3 cell line, cells were treated with 80 nM of MRCL3 siRNA in 1.5 mls of Opti-MEM media along with 30 µL of Lipofectamine 2000 Transfection Reagent (Invitrogen) in 1.5 mls of Opti-MEM media totaling 3 mls. The treated cells were

incubated at room temperature for 20 minutes. Upon completion of incubation, 2 mls of Optim-Mem medium was added and incubated over a course of 24,48, and 72 hours. After transfection of each hour increment, an addition of 6 mls of RPMI 1640 medium, supplemented with 10% fetal bovine serum, 2mM L-Glutamine, 100 U penicillin/0.1 mg/ml streptomycin was added to transfected cells. Cells were harvested and protein lysate was generated for future experiments.

Proliferation (MTS) assay

Cells were transfected with MRCL3 siRNA over the course of 48 hours. Cell viability was indirectly assessed using a colorimetric, 3-(4, 5-Dimethyl-2-thiazoyl)-2, 5-diphenyl-2H tetrazoliumbromide (MTS) assay. In brief summary, 5 x 10^3 mls of the BxPC3 cells were plated in Fisherbrand 96 well cluster dishes. After twenty-four hours of incubation, RPMI 1640 medium was removed and 40 µl of MTS solution was added to each well. The 96 well plates were placed in an incubator for 1-3 hours at 37°C in 5% CO2 and read at a wavelength of 490 nm on the Labsystems Multiscan Ascent plate reader.

Western blot analysis

Western blot analysis was performed using cells cultured at a density of 1.0 x106/ml. The cells were harvested and pelleted in a Eppendorf microcentrifuge tube (1,200 rpm, 5 minutes, 4 ° C), washed in 1X PBS and later lysed in 50 µL of lysis buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.5% deoxycholic acid, 1% nonidet-P40 (NP40), 0.1% SDS, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 100 µg/ml leupeptin. Protein concentration was measured using a Bio-Rad colorimetric protein assay kit (Bio-Rad). Protein was separated on SDS-polyacrylamide gels and transferred onto a nitrocellulose membrane (Schleicher and Schuell GmbH, Dassel, Germany) by electroblotting overnight in 25 mM Tris (pH 8.3), 192 mM glycine, 20% (v/v) methanol, 20V,100 mA at 4° C. After blocking with 10% (w/v) electrophoresis-grade biotin-depleted non-fat dry milk (BioRad) in 1X PBS and rinsed with 1X PBS, membranes were incubated with the primary antibodies. c-Fos antibody, c-Jun antibody and Caspase 3 antibody were used as primary antibodies. Horseradish peroxidase-conjugated anti-mouse antibody whole IgG were used as a secondary antibody for all three primary antibodies. The detection was accomplished using the enhanced chemiluminescence (ECL) detection system (Amersham Pharmacia Biotech GmbH, Freiburg, Germany).

Phospho p53 assay

BxPC3 cells were transfected with MRCL3 siRNA for 48 hours. Cells were harvested and centrifuged. An addition of 800 µl of 1X Dubecco's Phosphate Buffered Saline (PBS) and 200 µl of Formaldehyde were added to pellet and incubated at 37° C for 10 minutes. After 10 minutes, the pellet was placed on ice for 1 minute and later centrifuged. Cells were resuspended in 400 µL of 90% methanol and placed on ice for 30 minutes. After 30 minutes, 2 mls of incubation buffer was added to cells and centrifuged. Upon completion of centrifugation, 90 µL of incubation buffer was added to cells and incubated at room temperature for 10 minutes. Next, conjugate antibody phospho p53 dilution of 1:50 in 1X PBS was added to cells and incubated for 1 hour in a dark room at room temperature. Cells were washed with 2 mls of incubation buffer, centrifuged, and resuspended in 0.5 mls of 1X PBS. Florescence-activated cell sorting (FACS-Vantage) system (Becton-Dickinson, San Jose, CA) was used to analyze the samples.

Cell viability and Sub G1 apoptosis assay

Flow cytometry was used to assess Sub G1 DNA content in an entire cell population. 2 x 105 mls of cells were seeded in 24-well plates and cultured for 48 hours prior to treatment.

Cells were then transfected with MRCL3 siRNA over a course of 72 hours in Opti-MEM I Reduced Serum culture media. The cells were harvested by centrifugation, washed once with 1X PBS and suspended in 400 µl of propidium iodide (PI) solution (propidium iodide 50 µg/ml, 0.1% sodium citrate and 0.1% Triton- X 100). The Sub G1 DNA content was determined using a Florescence-activated cell sorting (FACS-Vantage) system (Becton-Dickinson, San Jose, CA).

Results

Verification of endogenous levels of MRCL3 protein expression

To investigate the role of MRCL3 in pancreatic cancer, cellular lysate of several pancreatic cell lines were generated. The cell lines generated were HPDE, Capan-1, Miapaca, BxPC3 and ASPC-1. Then, the intracellular distribution pattern of MRCL3 was examined by western blotting using an anti- MRCL3 antibody. These studies demonstrated that MRCL3, in accordance with other reports, was

expressed exclusively in HPDE and BxPC3 and not in Capan-1, ASPC-1 and Miapaca (Figure 1). BxPC3 was chosen because it is robust in MRCL3 expression in comparison to HPDE, which is a normal pancreatic cell line. The cytosolic marker, beta actin was used as a control to demonstrate equal loading of the lysate.

Verification of MRCL3 Knockdown in BxPC3 Cell Line and Transformed Phenotype In this context, next was determined if knockdown could alter long-term proliferation and clonogenic survival of BxPC3 cells by using western blot analysis. MRCL3 knockdown in BxPC3 cells resulted in a significant decrease in cell proliferation (Figure 2). It has been previously demonstrated by [5] that caspase-9 activation leads to the processing and activation of caspase 3. Additionally, caspase-3 appears to be an essential component of the apoptotic machinery in many cell types and causes activation of a cascade of executioner caspases leading to the demise of the cell as proven in previous studies done by [6]. The involvement of MRCL3 in caspase-3 processing was next examined. Western blot analysis showed that MRCL3 siRNA treated cells resulted in complete activation and processing of caspase-3 (Figure 3). This suggests that MRCL3 is necessary for maximal activation of caspase-3 in BxPC3 cells.

Status of MRCL3 Knockdown in BxPC3 cell line

Western blot analysis was also carried out to assess the status of gene knockdown over the course of 24, 48 and 72 hours. In this study, transfection of cells with MRCL3 siRNA resulted in a highly significant decrease in MRCL3 expression levels as judged by western blotting analysis without any alteration in β-actin expression (Figure 4). The gene knockdown was maintained for 2-3 days in cells treated with MRCL3 siRNA in comparison to control and scr siRNA, which were untreated.

MRCL3 Knockdown Promotes Activation of Phospho p53 in BxPC3 Cell Line p53 is a tumor suppressor gene known to be activated in response to DNA damage resulting in programmed cell death and/or cell cycle arrest. The Phospho p53 Assay was used to determine whether p53 is phosphorylated in the BxPC3 cell line upon MRCL3 silencing.

The loss of MRCL3 resulted in the phosphorylation of p53 in treated BxPC3 cell line with MRCL3 siRNA as indicated by a slight shift compared to the control (Figure 5). This outcome suggests MRCL3 plays a role in regulating p53 and promoting apoptosis in the BxPC3 cell line.

MRCL3 Induces Apoptosis in BxPC3 Cell Line.

In this study, cell death was examined using Sub G1 DNA content assay. BxPC3 cell line treated with MRCL3 siRNA displayed a substantial increase of cell death (10%) compared to scramble siRNA (4%) and control (5%) (Figure 6). This signifies that MRCL3 induces apoptotic activity in the BxPC3 cell line.

c-Fos/c-Jun Transcriptional Function in MRCL3 siRNA Treated BxPC3 Cell Line

To investigate MRCL3 mechanisms of action, the expression of AP-1 protein factors, c-Fos and c-Jun in BxPC3 cells was examined. Members of the Fos family dimerize with c-Jun to form the AP-1 transcription factor. This dimerization, in return, up-regulates transcription of a diverse range of genes involved from proliferation and differentiation to defense against invasion and cellular damage. In this study, it has been shown that MRCL3 knockdown in these cells increased the expression of c-Jun (Figure 7), while blocking c-Fos expression (Figure 8). These results imply that the MRCL3 pathway is mediated through AP-1 transcriptional factors. However, additional studies are required for verification.

Statistical analysis

Data was analyzed using SAS 9.1 software. Results showed a significant difference between control (1.89 ± 0.74) and MRCL3 siRNA treated BxPC3 cells (1.39 ± 0.48) with ($p = 0.0002$). However, control and scr siRNA (1.95 ± 0.70) were not significantly different as shown in (Figure 2).

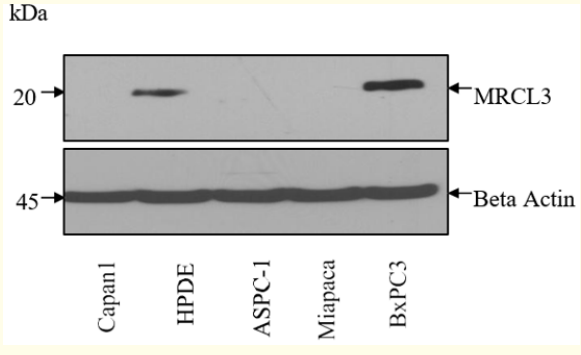

Figure 1: Verification of endogenous levels of MRCL3 in pancreatic cancer cells: Western blotting analysis of MRCL3 protein expression in various pancreatic cell lines: Capan-1, HPDE, ASPC-1, Miapaca, and BxPC3.

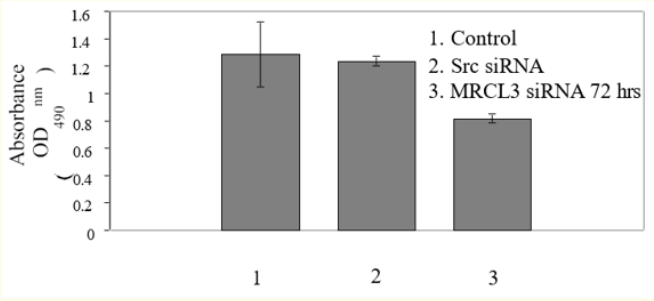

Figure 2: Effect of MRCL3 knockdown on cell proliferation in BxPC3 cell line: Graph showing BxPC3 cell viability was determined using MTS. P-value equals 0.0002.

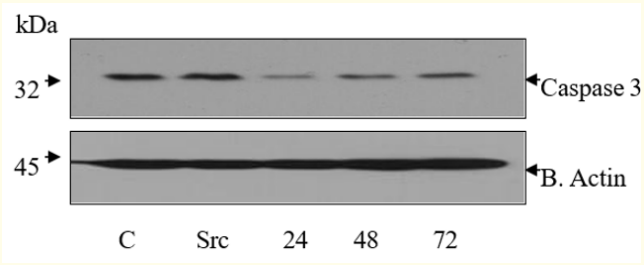

Figure 3: MRCL3 siRNA results in activation and processing of caspase 3: Western Blot analysis of caspase 3 in BxPC3 cells treated with MRCL3 siRNA versus control and scramble.

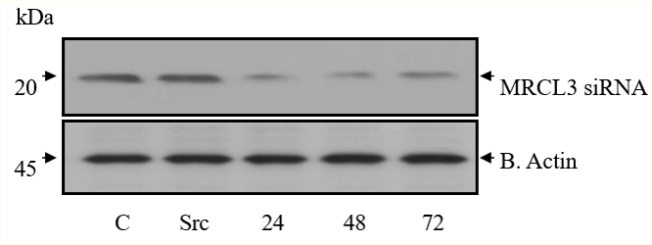

Figure 4: Status of MRCL3 knockdown in BxPC3 cell line: Western Blot analysis of MRCL3 knockdown: twenty- four, forty-eight, and seventy hours after MRCL3 siRNA transfections. Protein lysate was generated and analyzed.

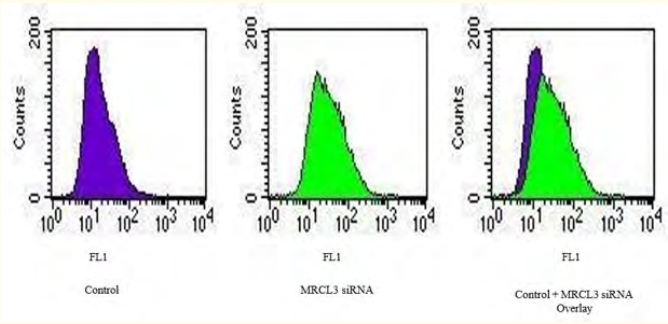

Figure 5: MRCL3 activates phospho p53 in BxPC3 cells: MRCL3 knockdown promotes activation of Phospho p53 in treated BxPC3 cell line with MRCL3 siRNA as indicated by slight shift in comparison to control. Cells were transfected with MRCL3 siRNA for 48 hours.

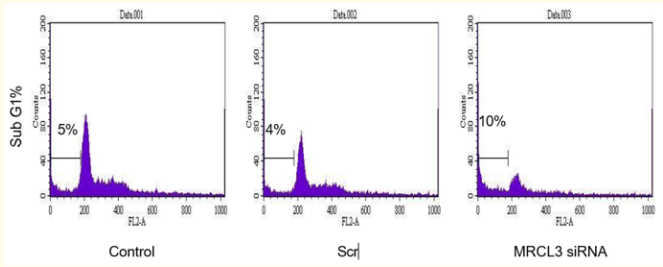

Figure 6: MRCL3 siRNA induces apoptosis in BxPC3 cells: Representative cell cycle histograms of BxPC3 cells treated with MRCL3 siRNA for forty-eight hours. Percent apoptotic cells are shown.

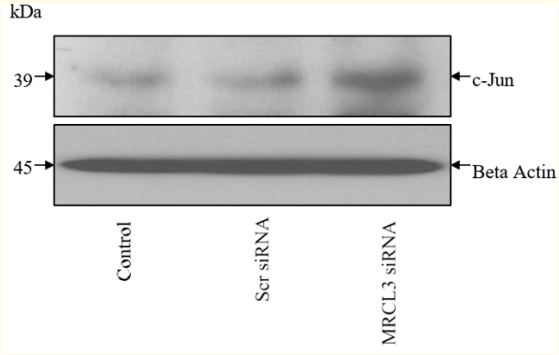

Figure 7: MRCL3 siRNA activates c-Jun protein expression: MRCL3 expresses c-Jun transcriptional function in BxPC3 cell line. Cells were treated with MRCL3 siRNA for 48 hours. MRCL3 siRNA treated BXPC3 cell line displayed c-fos expression.

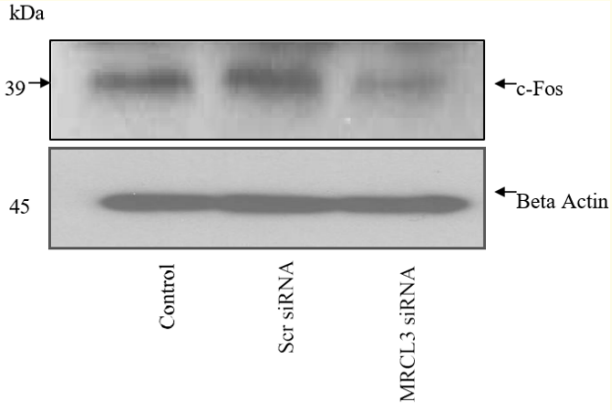

Figure 8: MRCL3 inhibits c-Fos protein expression in BxPC3 cells: MRCL3 expresses c-Fos transcriptional function in BxPC3 cell line. Cells were treated with MRCL3 siRNA for 48 hours.

Discussion

MRCL3 is a highly conserved, ubiquitously expressed regulatory protein with a molecular weight of 20 kDa that has been proven by [1] to interact with actin to generate the force for cellular movements and are implicated in cytokinesis, receptor capping, and cell locomotion. In this study, it has been demonstrated that MRCL3 is involved in mediating transcriptional factors, cell proliferation and apoptosis in pancreatic cancer.

In this study, a substantial increase of cell death was demonstrated by Sub G1 DNA content analysis in MRCL3 siRNA treated BxPC3 cell line in comparison to controls.

Previous research carried out by [7] has shown that p53 is a tumor suppressor gene. It later was known to be activated in response to DNA damage resulting in programmed cell death and cell cycle arrest by [8]. This study revealed that the loss of MRCL3 resulted in the phosphorylation of p53 in BxPC3 cell line treated with MRCL3 siRNA. This outcome suggested that MRCL3 plays a role in phosphorylating p53 and promoting apoptosis in the BxPC3 cell line. Together these findings demonstrated that MRCL3 plays a critical role in programmed cell death in the BxPC3 cell line. Next, whether the loss of MRCL3 had any impact in cell viability was questioned. Cell proliferation was verified by using the MTT assay. MRCL3 siRNA treated BxPC3 cell line inhibited cell proliferation by 10% in comparison to the controls.

This slower rate of proliferation suggested that MRCL3 plays a major role in cell viability of BxPC3 cell line. However, it is imperative to determine the role of MRCL3 in other pancreatic cell lines. It has been previously demonstrated by [5] that caspase-9 activation leads to the processing and activation of caspase 3. Additionally, caspase-3 appears to be an essential component of the apoptotic machinery in many cell types and causes activation of a cascade of executioner caspases leading to the demise of the cell as proven in previous studies done by [6]. In this study, caspase 3 processing caused the occurrence of apoptotic activity in the BxPC3 cell line treated with MRCL3 siRNA. Using western blot analysis, observations in this study are consistent with previous studies done by [6]. In this investigation, MRCL3 siRNA treatment in the BxPC3 cell line resulted in complete activation and processing of caspase-3. This suggests that MRCL3 is necessary for maximal activation of caspase-3 in BxPC3 cells. Studies by [9] have validated that AP-1 protein factors such as c-Fos and c-Jun have involvement in forming the AP-1 transcription factor when dimerized together. This dimerization, in return, up-regulates transcription of a diverse range of genes involved from proliferation and differentiation to defense against invasion and cellular damage as previous demonstrated by [10]. This study has shown that MRCL3 knockdown in BxPC3 cells increased the expression of c-Jun, while blocking cFos expression. These results imply that the MRCL3 mechanisms of action are mediated through AP-1 transcriptional factors.

There are limited cancer therapeutics that result in positive feedback in the case of pancreatic cancer. For these reasons, this study sheds several insights into possible targets of treatment that can inhibit tumor formation and growth. Overall, this study will help to progress perception of the mechanisms of action of MRCL3 in inhibiting pancreatic cancer cells.

Conclusion

From potential pancreatic cancer therapeutic targets and biomarkers, this study has identified a small set of the most promising regulatory candidates. Optimized meta analysis strategy was employed by using raw data from several independent pancreatic cancer transcriptome data sets. In this approach, the candidate genes generated for each dataset were combined and statistically evaluated to pinpoint genes that are differentially

expressed in pancreatic cancer compared to matched normal pancreas. From these results, MRCL3 emerged as top priority therapeutic target.

The selection of MRCL3 is based on: 1) Experimental in vitro and in vivo evidence supporting a causative relevance in the development and/or progression of cancer; 2) High frequency of overexpression and strong association with pancreatic cancer; 3) Restricted expression in normal tissues limiting expected overlap; 4) Overexpression in other cancers suggesting broad development potential. This study has successfully validated the role of MRCL3 in pancreatic cell line, BxPC3.

This study has revealed that MRCL3 mediates transcriptional factors such as c-Jun and c-Fos, thereby inhibiting the proliferation of BxPC3 cells. This inhibition was associated with the activation and processing of caspase 3 that plays a major role in execution of apoptosis. To further evaluate the role of MRCL3 in apoptosis, Sub G1 DNA content assay was executed. BxPC3 cells treated with MRCL3 siRNA revealed a drastic increase in the levels of cells undergoing apoptosis by 10%. Overall, this study will help to progress our perception of the mechanisms of action of MRCL3 in inhibiting pancreatic cancer cells.

Acknowledgements

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Conflict of Interest

None.

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