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Chmp1 is Implicated in the Development of Pancreatic Tumor Via the Retinoic Acid Signaling Pathway

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CHMP1 IS IMPLICATED IN THE DEVELOPMENT OF PANCREATIC TUMOR VIA THE RETINOIC ACID SIGNALING PATHWAY

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ABSTRACT

In the present study, we investigated the involvement of Chmp1 (Chromatin Modifying Protein 1/Charged Multivesicular body Protein 1) in the development of mouse pancreatic acinar tumor cell line. CRL 2151 cell line was transfected with Chmp1/CS2 vector to compare growth, morphology and expression of Chmp1, p53 and pp53 (ser 37) with control-transfected cells. CRL 2151 cells were treated with all-trans retinoic acid (ATRA) to compare growth, morphology and expression of Chmp1 and p53 with control-treated cells. Strabismus was used as control. Results showed inhibition of growth but no morphological change in transfected cells. Western blot analysis showed that Chmp1 transfection upregulated the expression of p53, pp53 and Stbm temporarily. Treatment of cells with ATRA did not inhibit growth or show morphological change. However, Western blot analysis demonstrated the upregulation of Chmp1, p53 and Stbm proteins. Microarray analysis of samples transfected with Chmp1 or treated with retinoic acid was done to determine if the same set of genes will be regulated. The pro-apoptotic genes Bad and Bak are among the genes up-regulated. Cell division cycle protein, Cdca7, was among the genes down-regulated. Results from Western blot analysis confirmed the expression of Bad and Bak genes. Taken together, these results suggest that Chmp1 functions in the suppression of pancreatic tumor by the retinoic acid signaling pathway.

Key Words: CRL 2151, Chmp1, Stbm, p53, Bad, Bak, Cdca7, ATRA.

DEDICATION

This work is dedicated to the loving memory of my parents, Samuel and Fehintola Arowosaiye.

ACKNOWLEDGMENTS

Foremost, I gratefully acknowledge Dr. Maiyon Park for the guidance and support she gave throughout the completion of this work. Many thanks to Dr. Simon Collier for all the supports and help rendered towards the successful defense of this work. I also wish to thank him and Dr. Gho-Zhang Zhu for their valuable suggestions to the manuscript. I would like to thank Dr. Goran Boskovic for carrying out the microarray analysis aspect of this work, and Dr. Natalia Belogortseva for her useful suggestions at various stages of my investigation. Brandon Orr, Kayla White and Kristy Dillon all deserve my thanks for helping in correcting and proof-reading the thesis. Margaret McFarland assisted with the scanning of results from Western-blot analysis, thank you. To my good friend Vani Uddemarri (M.D) and Kevin Rice who introduced me to Western-blot assay, I say thanks. I also wish to appreciate the kindness and friendliness shown to me by Susan Weinstein, Vickie Craiger and Mary Jo Smith throughout my studentship in the department of Biological Sciences. Finally, I thank the NIH whose COBRE grant to Dr. Park enabled me to carry out this work.

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LIST OF SYMBOLS

ATRA	All-trans retinoic acid
CHMP	Chromatin Modifying Protein; Charged Multivesicular body Protein
Stbm	Strabismus
CRL2151	Mouse pancreatic acinar carcinoma cell line
RAR	Retinoic acid receptors
RXR	Retinoid X receptors
MVB	Multivesicular body
PcG	Polycomb-group
Pcl	PcG protein Polycomblike
SAP7	Strabismus associated protein 7
MAb	Mouse monoclonal antibody
DMSO	Dimethyl sulfoxide
RB	Retinoblastoma
RA	Retinoic acid
ESCRT-III	Endosomal Sorting Complex Required for Transport-III
HSP	Hereditary Spastic Paraplegia
SAL1	Supernumerary aleurone layers 1
Cdca7	Cell division cycle associated protein 7
HeLa	Human epithelial carcinoma cell line
SKD1/VPS4	Vacuolar protein sorting 4b

COS7	African green monkey kidney cell line
HEK 293	Human embryonic kidney cell line
PC12	Pheochromocytoma cell line
PCP	Planar Cell Polarity

CHAPTER 1

INTRODUCTION

Chmp1 (Chromatin Modifying Protein 1/Charged Multivesicular body Protein 1) is a novel protein found to be widely conserved across eukaryotes [1, 2]. It is localized in the nucleus as well as in the cytoplasm. It is found in the interphase nuclear matrix and mitotic chromosome scaffold affecting chromatin structure and cell cycle progression [2-4]. In the cytoplasm, it is involved in protein sorting to the multivesicular body [1, 4-7]. Chmp1 therefore plays a dual function of nuclear gene regulation and membrane trafficking.

Very few studies have so far been done on Chmp1. One of the studies reported that Chmp1 localizes to early and late endosomes in HEK 293 (human embryonic kidney) and COS7 (African green monkey kidney) cell lines [1]. This study further showed that it interacts with SKD1/VPS4 (vacuolar protein sorting 4b) in sorting proteins into multivesicular bodies. Another investigation by Stauffer et al, (2001) identified Chmp1 as a binding partner of Pcl (PcG polycomblike) proteins in HEK 293 cell line. The PcG (polycomb group) are proteins involved in gene silencing. In addition, they reported that Chmp1 affects nuclear structure by increasing nuclear DNA concentration through chromatin condensation. It was shown to also affect DNA replication by arresting cells in S-phase of the cell cycle.

A closely related protein family of Chmp1 (Chmp1b) was studied by Reid et al, (2004). They identified Chmp1b as a binding partner of Spastin in COS7 and PC12

(Pheochromocytoma) cell lines. Spastin is a protein involved in hereditary spastic paraplegia (HSP), a neurological disorder characterized by progressive weakness and stiffness of the leg. They found that the expression of Chmp1b prevented the development of the abnormal phenotype associated with the expression of mutated *spastin*, suggesting a regulatory role for Chmp1b. Furthermore, they suggested that Chmp1b interacted with Spastin to play a role in membrane trafficking. Interestingly, a homolog of Chmp1 was identified in plant. This homolog, SAL1 (supernumerary aleurone layers 1), was studied in maize by Shen et al, (2003). They found SAL1 to be involved in regulating the formation of aleurone cell layer in maize grains. Failure to target internalized membranes to lytic vacuoles in grain with mutated *sal1* resulted into more layers of aleurone cells in defective maize endosperm. These results suggest that SAL1 may be involved in membrane trafficking and the lack of this function may contribute to abnormal growth.

Even though Chmp1 has been implicated in cell cycle progression, chromatin structure, gene silencing and membrane trafficking, its function in vertebrate development and in tumor formation is not yet known.

Our lab identified Chmp1 as one of Strabismus (Stbm) binding proteins from yeast two-hybrid screen. Strabismus is known to function in the Planar Cell Polarity (PCP) signaling pathway, modulating convergent extension movement in zebrafish [8]. Mis-expression of Chmp1 produced convergent extension movement defects in zebrafish similar to its binding partner, Stbm [Park et al, in preparation]. However unlike Stbm, Chmp1 mis-regulation (either by microinjecting embryo with Chmp1 mRNA or

Morpholino) induced hyperplasia (Figure 1), an abnormal cell growth, in zebrafish indicating that Chmp1 may function in tumorigenesis [Park et al, in preparation].

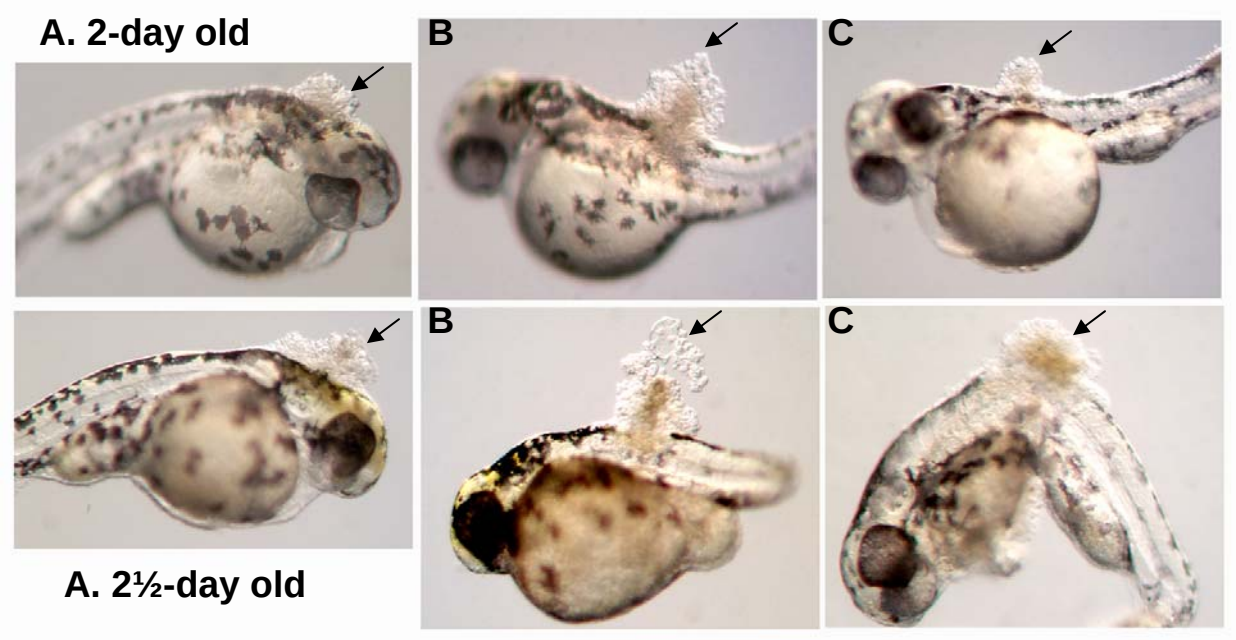

Figure 1: Induction of hyperplasia in zebrafish by Chmp1. Embryo was microinjected with Chmp1 mRNA, mis-regulation of Chmp1 induced abnormal growth. The embryos on the top are zebrafish at 2 days old; the images on the bottom are the same fish at 2½ days (Courtesy of Dr. Park's lab).

To test this hypothesis, a human cancer profiling array from CLONTECH cancer Profiling Array II was screened to measure Chmp1 mRNA level. Also, a human cancer tissue array was screened to quantify Chmp1 protein level. Results obtained from the profiling array showed differential expression of Chmp1 between the various tumors and

their corresponding normal tissues. The pancreas showed the greatest differential expression between the tumors and their normal tissues (Figure 2).

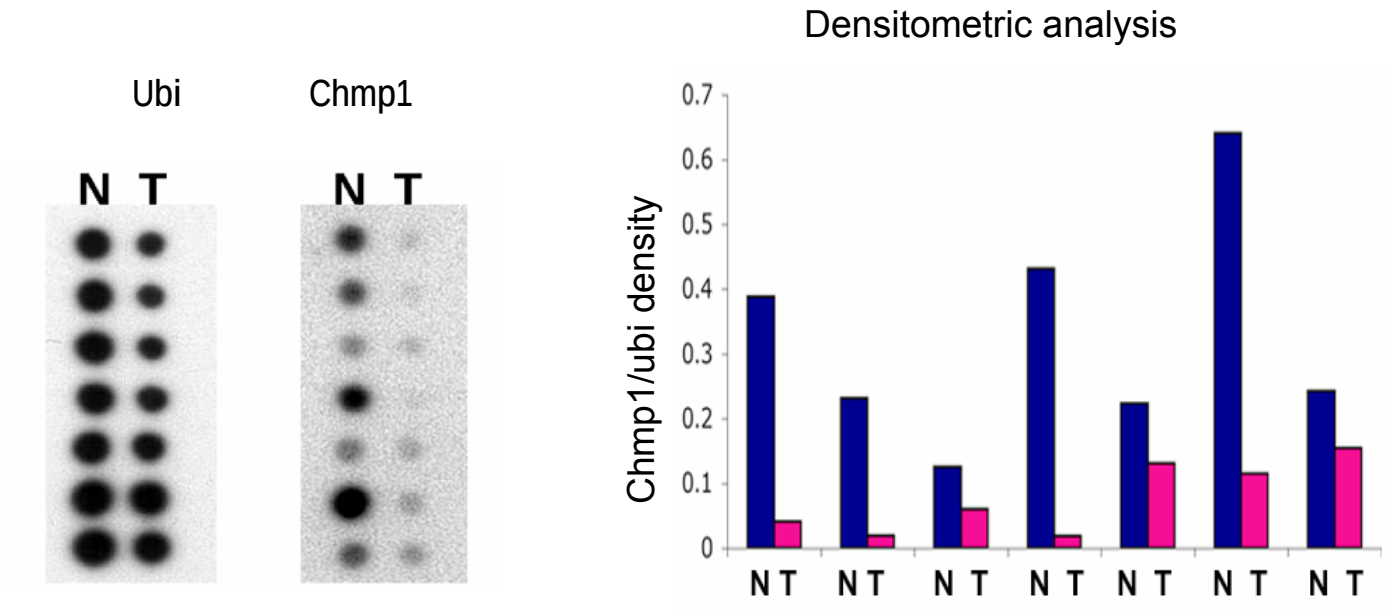

Figure 2: Cancer profiling array of cDNAs from various pancreatic tumors and their corresponding normal tissues. The left shows control Ubiquitin mRNA expression from various normal (N) and pancreatic tumor (T) samples. The array on the right is the same array showing Chmp1 mRNA level. The graph is a densitometric analysis of the results. This shows the dramatic repression of Chmp1 in pancreatic tumor (Courtesy of Dr Park's lab).

Among the pancreatic cancer tissues investigated, pancreatic acinar tumor is the only tumor which showed almost no expression of Chmp1 protein. Chmp1 protein expression within the pancreatic acinar tumor cells was greatly reduced even though its expression in the connective tissue cells was not reduced (Figure 3).

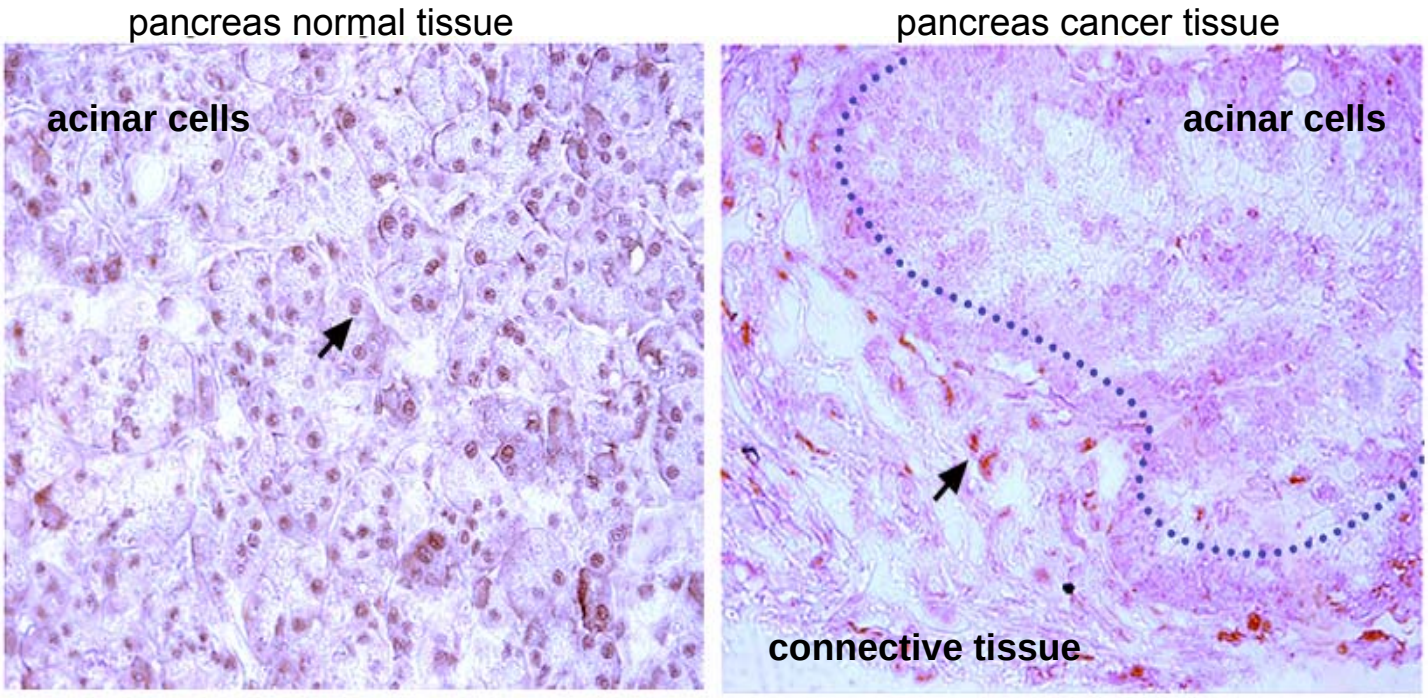

Figure 3: Chmp1 antibody staining. Normal and tumor cells of the pancreas were immunostained with Chmp1 antibody. Arrows point to Chmp1 protein (dark brown stain) within the nucleus of the cells. Chmp1 protein expression within the pancreatic acinar cells (right) is greatly reduced even though its expression in the connective tissue cells is not reduced (Courtesy of Dr. Park's lab).

On the basis of these results, we decided to investigate the role that Chmp1 may play in the development of pancreatic acinar tumors. Our lab is the first to investigate the involvement of Chmp1 in convergent extension movement and the induction of hyperplasia in vertebrate development. It is also the first to investigate the expression of Chmp1 during cancer development.

Retinoids are natural and synthetic derivatives of vitamin A (retinol). They exert dramatic effects on cell death and differentiation in human tumor cells, and in vertebrate embryogenesis; modulating differentiation, proliferation and apoptosis in both normal and neoplastic tissues [9-15]. Several investigators have demonstrated that all-trans retinoic acid (ATRA) inhibits growth of pancreatic cancer cells both *in vivo* and *in vitro* [9, 13, 14, 16, 17]. This inhibition is thought to occur in part through the p53, a tumor suppressor, pathway [18].

Pancreatic cancer is one of the rarest cancers and yet it is the fifth leading cause of cancer death in the United States, with an annual of about 28,200 diagnoses and death [19-21]. The mortality rate is the highest among cancer types, in part because of the asymptomatic nature of the disease in early stages [21]. It affects men twice as frequently as women and is more likely to develop after the age of 40 [22]. Pancreatic cancer risks increase with chronic pancreatitis, diabetes mellitus, genetic factors (more common in African Americans than Caucasians), smoking, excess alcohol consumption, high-fat diets, and exposure to industrial chemicals such as urea, naphthalene or benzidine. Symptoms include weight loss, abdominal pain, and nausea, loss of appetite, itching, jaundice, and constipation. Because its symptoms mimic many other common health problems [22], it often goes undetected until it is too late to treat effectively.

Pancreatic exocrine tumors are more common than the tumors from the endocrine. The majority of the exocrine tumors are adenocarcinomas, and nearly all of these are ductal adenocarcinomas. Pancreatic acinar cell carcinomas are rare neoplasms of the exocrine pancreas, comprising less than 1% of primary pancreatic tumors[23, 24]. They are distinct from the more common pancreatic ductal

adenocarcinomas. The prognosis in adults is poor, with the majority of patients showing evidence of metastatic disease either at or subsequent to diagnosis [23, 25-28] ; and a reported mean survival of 18 months[23, 29, 30].

Pancreatic cancer is the only cancer with a diagnosis rate less than the mortality rate. More studies are therefore needed to better understand and address the cellular mechanisms and pathways by which this cancer develops. In the present investigation, we hypothesized that Chmp1 may function through retinoic acid signaling to inhibit cell growth by activating p53 [18].

PURPOSE

Our long term goal is to identify the potential pathways by which Chmp1 may function in pancreatic tumors. The first purpose was to determine how Chmp1 over-expression or gain of function through transfection may affect the growth of CRL2151, a pancreatic acinar tumor cell line. Morphology of cells was observed under the microscope and the number was counted. Based on the report from several investigators that retinoic acid inhibits growth of pancreatic cancer cells, we chose to treat CRL2151 cells with ATRA. The second purpose was to investigate growth inhibition, morphological changes and expression level of Chmp1, p53 and Stbm in CRL2151 cells after treatment with ATRA. We chose p53 on the basis of report of studies that indicates p53 to be expressed during cancer growth inhibition and its stability by phosphorylated p53 (pp53). Stbm was chosen as a control on the basis of it being a binding partner of Chmp1 in our previous investigation. The third purpose was to determine if Chmp1 gain of function or over expression would induce the up-regulation of p53 in CRL2151 cells. Fourthly, we set out

to confirm that Chmp1 functions through retinoic acid by activating p53. In this case we carried out microarray analysis of samples transfected with Chmp1 or treated with retinoic acid to determine if the same set of genes will be regulated. The rationale for this research was that if the function of Chmp1 in pancreatic tumor is known, Chmp1 may serve as a pharmacological target protein for treating pancreatic tumors.

SPECIFIC AIMS

The objective of this study was to determine the role of Chmp1 during the development of pancreatic acinar tumor. The hypothesis is that Chmp1 may function through retinoic acid signaling to inhibit cell growth by activating p53. In this study, we determine the effect of exogenous Chmp1 and ATRA on growth, morphology and expression of Chmp1, p53, pp53 and Stbm of CRL2151 cell line. We also determine the genes expressed by these two treatments.

Specific Aim 1:

To determine the effect of Chmp1 on the growth and morphology of CRL2151. In this case we transfected cells with Chmp1/CS2 vector and CS2 vector (control) for 1, 2, 3 and 4 days. Transfection with Stbm/CS2 vector also served as control. Photos of cells were taken, followed by cell counting and statistical analysis of cell numbers.

Hypothesis:

We hypothesized that Chmp1 would induce a decrease in cell growth and cause morphologyical changes.

Specific Aim 2:

To determine the effect of ATRA on cell growth, morphology and the expression of Chmp1, p53 and Stbm in CRL2151 cells. Cells were treated with ATRA in Dimethyl Sulfoxide (DMSO) for 1, 2, 3 and 4 days. Photos of cells were taken. Cells were trypsinized for cell count and lysed for Western blot on days 1, 2, 3 and 4. Cell numbers were statistically analysed and alterations in the expression of Chmp1, p53 and Stbm were evaluated by densitometry method.

Hypothesis:

We hypothesized that ATRA will induce a decrease in cell growth and cause morphological changes in cells, with a corresponding increase in the expression of Chmp1 accompanied by increase in the expression of p53.

Specific Aim 3:

To determine the level of expression of Chmp1, p53, pp53 and Stbm in CRL2151 cells transfected with Chmp1. Cells were transfected with Chmp1/CS2 and CS2 vectors for 1, 2 and 3 days. Transfection of cells with Stbm/CS2 vector also served as control. Cells were trypsinized and lysed for Western blot. Alterations

in the expression of Chmp1, p53, pp53 and Stbm were evaluated by densitometry.

Hypothesis:

Our hypothesis was that the level of Chmp1 expression will increase with an accompanied increase in p53 and pp53 expression.

Specific Aim 4:

To determine that Chmp1 functions through retinoic acid by activating p53. In this case we expect that the same genes will be up or down regulated when CRL2151 cells are transfected with Chmp1 or treated with ATRA. To examine this possibility, we treated cells with ATRA and also transfected cells with Chmp1. Cells were trypsinized and total cellular RNA was prepared using Tris Reagent. The gene expression level was determined by microarray analysis. Gene expression was confirmed by Western-blot. Expression of proteins was evaluated by densitometry.

Hypothesis:

We hypothesized that similar genes will be expressed when CRL2151 cells are treated with ATRA and transfected with Chmp1.

CHAPTER 2

REVIEW OF THE LITERATURE

INTRODUCTION

This chapter presents a review of the relevant literatures concerning the present study. Specifically, the following areas will be addressed: 1) the potential functions of Chmp1 and 2) the role of retinoids and their inhibition in the growth of cancers.

The potential functions of Chmp1

Chmp1 is a novel protein and a member of the Chmp protein family. It is known to contain a predicted bipartite nuclear localization signal. Also, it is reported to distribute to the nuclear matrix and to the cytoplasm [2]. In the nucleus, Chmp1 is localized in the nuclear matrix of HeLa (human epithelial carcinoma) cells where it affects chromatin structure and cell-cycle progression [2]. Chmp1 also functions as a member of a newly defined family of vesicle trafficking proteins in the cytoplasm in yeast [1, 4-7].

A zebrafish protein was identified from yeast two-hybrid screen, and named as SAP7 (Strabismus Associated Protein 7) in our lab. This protein was found to be a homolog of human Chmp1. The interaction between Chmp1 and Strabismus (Stbm) has also been confirmed by co-immunoprecipitation in our lab. Strabismus is involved in convergent extension movements in vertebrate [8, 31, 32]. Chmp1 also functioned in embryo development similar to Strabismus, and it induced hyperplasia when mis-regulated in zebrafish [Park et. al., in preparation] (Figure 1).

Several investigators have reported that the Chmp family of proteins are components of endosomal sorting complex required for transport-III (ESCRT-III)[3, 4, 7, 33, 34]. ESCRT-III is a membrane-associated protein complex that mediate the sorting of cargoes to the multivesicular body. This is why Chmp are referred to as Charged Multivesicular body Proteins, in addition to being called Chromatin Modifying Proteins. The Chmp proteins were originally identified in yeast genetic screens[35, 36]. Subsequent studies have identified at least one human homolog for each protein[37], indicating that they are conserved across eukaryotes. These proteins are recruited to sites of multivesicular body protein sorting and vesicle formation[34].

Proteins targeted for lysosomal degradation are sorted into vesicles that bud into late endosomal compartments called multivesicular bodies (MVB). Once formed, multivesicular bodies can undergo several different fates. They may either serve as long-term storage compartments, fusing with lysosomes to deliver the internal vesicles and their contents for degradation. Or they may fuse with the plasma membrane to release the vesicles as extracellular “exosomes.” MVB function in a number of important biological processes, including receptor down-regulation, antigen presentation, intercellular communication, and development[35, 38-40]. Vesicle trafficking therefore provides communication and transport between membrane-bound compartments involved in biosynthesis, degradation and cell surface signaling. Also, a number of enveloped RNA viruses, including HIV-1, are reported to usurp cellular proteins involved in MVB biogenesis to facilitate virus budding [37, 41].

Chmp1, like most other members of the Chmp family, is implicated in multivesicular body (MVB) formation. In this process it is reported to localize to early and late endosomes physically interacting with SKD1/VPS4 (vacuolar protein sorting 4b), a highly conserved protein directly linked to multivesicular body sorting in both human and yeast[33, 42-45].

Investigation by Stauffer, et al., (2001) revealed other important functions of Chmp1. They reported that Chmp1 has potent affects on nuclear structure by increasing nuclear DNA concentration through chromatin condensation. Also Chmp1 affects DNA replication by arresting cells in S-phase of the cell cycle. Furthermore they identified Chmp1 as a partner of the PcG protein polycomblike (Pcl)**.** The PcG (polycomb group) consists of a large, structurally diverse set of nuclear proteins, which functions both to select target genes and regulate their transcription in epigenetic modifications. The PcG proteins are involved in maintenance of the silenced state of several developmentally regulated genes. When Chmp1 is over-expressed, it is reported to encapsulate proteins in the Polycomb complex and localize them to visibly condensed chromatin, suggesting that Chmp1 may function with PcG in gene silencing [2].

Closely related family of Chmp1, Chmp1b, is identified as a binding partner of Spastin in COS7(African green monkey kidney) and PC12(Pheochromocytoma) cell lines [3]. Mutations in *spastin* are responsible for hereditary spastic paraplegia (HSP), a group of inherited disorders characterized by progressive weakness and stiffness of the legs. Expression of Chmp1b prevented the development of this abnormal phenotype associated with the expression of mutated *spastin*, suggesting a regulatory role for Chmp1b. Also, Chmp1b interacted with Spastin to play a role in membrane trafficking.

Another homolog of Chmp1, SAL1 (supernumerary aleurone layers 1), is identified in maize [6]. SAL1 protein is involved in the regulation of aleurone cell (a component of the endosperm in cereal grains) formation in maize grains. The failure to target internalized membranes to lytic vacuoles in grain carrying mutated *sal1* caused more layers of aleurone cells in defective maize endosperm, suggesting that SAL1 May be involved in membrane trafficking.

Overall, reports from the few studies on Chmp1 suggest that Chmp1 may function in several pathways which involve gene regulation and membrane trafficking. Even though Chmp1 has been implicated in cell cycle progression, chromatin structure, gene silencing and membrane trafficking, the function of Chmp1 in vertebrate development and in tumor formation has not been studied.

Summary

Chmp1 is a novel protein and a member of the Chmp protein family. Chmp1 is conserved across eukaryotes. It functions in gene expression and silencing. It also functions in cell-cycle progression. Chmp1 induces hyperplasia in zebrafish and is involved in convergent extension movement in embryogenesis. It is involved in protein sorting to the multivesicular body. Its function in tumors is yet unknown.

Retinoids and their inhibition in the growth of cancers

Retinoids belong to a class of molecules that are structurally related to vitamin A. They have potent anti-proliferative and differentiating effects on a variety of normal and neoplastic tissues [9, 10, 12-15]. They also play a crucial role in embryogenic

development [10, 12, 46-48]. They are therapeutically effective in the treatment of some cancers, skin disorders such as acne, psoriasis and photoaging, and have the potential for protecting certain cells from particular injury [49]. Retinoids have the ability to arrest growth in carcinoma cells, melanoma, lymphoma, neuroblastoma, and embryonic stem cells [50]. The potential role of different types of retinoids for pancreatic cancer *in vitro* and *in vivo* has been reported [9, 16, 51, 52], with several investigators demonstrating that all-*trans* retinoic acid (ATRA) inhibits growth of human pancreatic-cancer cells *in vitro* and *in vivo* [9, 16].

Cancers of the pancreas can be grouped according to where in the pancreas the cancer is or according to the type of cell the cancer has originated from**.** It can occur in the head, body or tail of the pancreas. The vast majority of cancers of the pancreas are the exocrine type, and most of these are adenocarcinomas, nearly all are ductal adenocarcinomas. The ducts carry pancreatic digestive juices to the main pancreatic duct and the duodenum. The rarer types of exocrine pancreatic cancers are cystic tumors, cancer of the acinar cells and sarcomas. The acinar cells are the cells at the ends of the ducts that produce the pancreatic juices.

Most of the effects of retinoids in pancreatic cancers are due to the activation of the retinoic acid receptors. This triggers transcriptional events that lead either to transcriptional activation or repression of retinoid-controlled genes. These biological effects of retinoic acid (RA) are mediated by nuclear retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Each receptor family consists of three receptor subtypes (α, β, γ) encoded by independent genes [50, 53, 54]. Modulation of gene expression occurs

through binding of ligand-activated RAR/RXR to retinoid responsive elements located in the regulatory regions of target genes [47, 55-62].

The inhibition of cancer cells by retinoids is thought to occur through the p53 pathway [18]. Inhibition may occur by means of G1-phase cell-cycle arrest resulting from the prevention of RB (retinoblastoma) phosphorylation and the up-regulation of p21 and p27, as well as the induction of apoptosis [17].

The p53 protein is a tumor-surpressor of cells that prevents their entry into the route to carcinogenesis[63]. It is a multifunctional protein that can modulate genes involved in the control of cell growth and apoptosis [64]. In response to cellular stress such as DNA damage, hypoxia and oncogenic signals, p53 functions in cell cycle progression checkpoints arrests[63, 65]. The protein p53 induces apoptosis (a type of programmed cell death) by target gene regulation and by translocating to the mitochondria to promote the release of cytochrome c [65, 66]. Commitment of cells to apoptosis is controlled largely by the interaction between members of the Bcl-2 protein family. Activated p53 tansactivates pro-apoptotic genes such as Bad and Bak [67]. Bad and Bak in turn trigger apoptosis when they bind to Bcl-2 or its homologs and block their antiapoptotic activity[67-71].

Several investigations on the effect of retinoids on pancreatic tumor cells have been done. It is not known if Chmp1 expression occurs when pancreatic tumor cells are treated with retinoids.

Summary

Retinoids modulate cellular proliferation and differentiation in a variety of tissues of the adult organism as well as during embryogenesis and development. Studies have been done to investigate RA-mediated growth inhibition and apoptosis on some pancreatic cancer cells. However no study has investigated whether Chmp1 functions through RA signaling.

CHAPTER 3

CHMP1 IS IMPLICATED IN THE DEVELOPMENT OF PANCREATIC TUMOR VIA THE RETINOIC ACID SIGNALING PATHWAY

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ABSTRACT

In the present study, we investigated the involvement of Chmp1 in the development of mouse pancreatic acinar tumor cell line. CRL 2151 cell line was transfected with Chmp1/CS2 vector to compare growth, morphology and expression of Chmp1, p53 and pp53 (ser 37) with control-transfected cells. CRL 2151 cells were treated with all-trans retinoic acid (ATRA) to compare growth, morphology and expression of Chmp1 and p53 with control- treated cells. Strabismus was used as control. Results showed inhibition of growth but no morphological change in transfected cells. Western blot analysis showed that Chmp1 transfection upregulated the expression of p53, pp53 and Stbm temporarily. Treatment of cells with ATRA did not inhibit growth or show morphological change. However, Western blot analysis demonstrated the upregulation of Chmp1, p53 and Stbm proteins. Microarray analysis of samples transfected with Chmp1 or treated with

retinoic acid was done to determine if the same set of genes will be regulated. The pro-apoptotic genes Bad and Bak are among the genes up-regulated. Cell division cycle protein, Cdca7, was among the genes down-regulated. Results from Western blot analysis confirmed the expression of Bad and Bak genes. Taken together, these results suggest that Chmp1 functions in the suppression of pancreatic tumor by the retinoic acid signaling pathway.

INTRODUCTION

Pancreatic cancer is the fifth leading cause of cancer death in North America [72]. It is second only to colon cancer as a cause of death from gastrointestinal malignancy, and affects men twice as frequently as women with more possibility of developing after the age of 40 [22]. It is the only cancer with a diagnosis rate less than the mortality rate. With resection surgery, radiotherapy and conventional chemotherapy, the prognosis of advanced pancreatic cancer has not recorded any significant improvement over the last 30 years, showing a 5-year survival rate of less than 5% [73]. However, several investigators have demonstrated that all-*trans* retinoic acid (ATRA) inhibits growth of human pancreatic-cancer cells *in vitro* and *in vivo* [9, 16].

Retinoids are vitamin A derivatives, used effectively in the therapeutic treatment of some cancers[9, 49]. Their effects on both normal and cancer cells include modulations of differentiation, proliferation and apoptosis [10, 15]. RARs and RXRs are the nuclear receptors of retinoids. They function as transcription factors modulating the activity of several target genes [10, 55].

Albrechtsson et al., (2002) studied the expression of retinoic acid receptors (RAR) in pancreatic cancer cells and the effect of three different retinoids on the cell number *in vitro*. They reported a decrease in cell number to high concentration of RA and the expression of two or more subtypes of RAR in all pancreatic cell lines investigated. Bold et al., (1996) studied the effect of retinoic acid on the growth of both exocrine and endocrine pancreatic cancer cell lines. The growth of all tumor cell lines was inhibited by retinoic acid. In their own investigation, Rosewicz et al., (1995) found a

time-and dose-dependent growth inhibition in pancreatic carcinoma cells resulting from retinoid treatment. There was expression of retinoid receptors and induction of differentiation in cells. Also the effect of TAC-101, a novel retinoid, was studied on 4 human pancreatic-cancer cell lines by Fujimoto et al., (1999). It was reported that TAC-101 inhibits the growth of certain pancreatic cancer cells by means of G1-phase cell cycle arrest due to the reduction of RB phosphorylation and the up-regulation of p21 and p27. It also induces apoptosis.

Another investigation involving retinoic acid-responsive protein was carried out by Kaiser et al., (1999). They identified the kinesin-related novel protein HsEg5 as an all-trans-retinoic acid (ATRA)-responsive gene in pancreatic carcinoma cells, mediating growth inhibition. The protein HsEg5 was found to play an essential role in spindle assembly and spindle function, resulting in a delayed progression through mitosis.

Even though various studies have demonstrated that retinoids inhibit the growth of pancreatic cancer cells, there is need for more studies that will provide a conceptual framework to identify novel therapeutic target proteins.

Chmp1 is a novel protein conserved in both complex and simple eukaryotes [2]. Howard et al., (2001) reported this protein to early and late endosomes in African green monkey kidney (COS7) and human embryonic kidney (HEK) cells. It was also found to physically interact with SKD1/VPS4, a highly conserved protein linked to multivesicular body sorting in yeast. They found Chmp1 overexpression to dilate endosomal compartments. Investigation of Chmp1 in HEK 293 cell line by Stauffer et al., (2001) also showed that when overexpressed, Chmp1 localizes to regions of condensed

chromatin. It recruits a polycomb group (PcG) protein to the condensed chromatin and can cooperate with co-expressed vertebrate PcG protein polycomblike (Pcl). In addition, Chmp1 has potent effects on nuclear structure and DNA replication. Their results suggest Chmp1 plays a role in stable gene silencing and DNA regulation.

Investigation from our lab showed that Chmp1 and its associated protein (Strabismus) produced convergent extension movement defects when overexpressed in zebrafish (Figure 1). It was also observed that Chmp1 induces hyperplasia, indicating Chmp1 may function in tumor formation [Park et al., in preparation]. To test this hypothesis, human cancer profiling array was screened to measure Chmp1 mRNA level. Human cancer tissue array was also screened to quantify Chmp1 protein level. Results showed differential expression of Chmp1 between the various tumors and their corresponding normal tissues. Interestingly, Chmp1 mRNA level was dramatically down-regulated in pancreatic tumor compared to the normal tissue (Figure 2). Among the pancreatic cancer tissues investigated, pancreatic acinar cancer cell is the only cell which showed almost no expression of Chmp1 protein (Figure 3).

The objective of this study was to investigate the molecular function of Chmp1 in the development of pancreatic tumors. We hypothesized that Chmp1 may function through retinoic acid signaling to inhibit cell growth by activating p53 in pancreatic cancer cells. In this study we investigated the effect of Chmp1 on the growth of CRL2151, a pancreatic acinar tumor cell line. Our results indicate that Chmp1 may function as a tumor suppressor in pancreatic tumors downstream of retinoic acid signaling pathway. Therefore, in addition to the reports that Chmp1 is involved in

nuclear gene regulation and cell cycle progression [2, 4], suggesting that it functions in DNA transcription and cell growth, Chmp1 may also function in tumor suppression.

MATERIALS AND METHODS

Chemicals

All-trans-Retinoic acid (ATRA) was obtained from Sigma. It was dissolved in Dimethyl Sulfoxide (DMSO, Fisher) at a concentration of 20μM prior to use.

Cell line and Cell Culture Condition

Mouse pancreatic acinar tumor cell line, CRL2151, was obtained from ATCC (America Type Culture Collection). Cells were maintained in HYQ Dulbecco's Modified Eagle's (DME) High glucose medium plus10% FBS and antibiotics (10000 units/ml penicillin G sodium and 10000 μg/ml streptomycin sulfate in 0.85% saline) at 37°C in a humidified atmosphere composed of 95% air and 5% CO2.

Cell Transfection

Cultured cells were harvested by trypsin digestion and seeded at 800,000cells/10cm plate in DME High glucose medium with 10% FBS for 24 hr. Human Chmp1 and Stbm genes were each previously cloned into CS2 vector containing CMV and SP6 promoters. The constructs were named Chmp1/CS2 and Stbm/CS2. CRL2151 cells were transfected with 5μg of Chmp1/CS2 or Stbm/CS2 vector in a 10cm plate, using Lipofectamine™ Reagent (Invitrogen). Cells were also transfected with CS2 vector only. Both Stbm/CS2 and CS2 transfections served as controls. The transfected cells were incubated for 1, 2, 3 and 4 days. Photos were taken from plates daily using Leica DMI6000B inverted microscope attached to DFC camera to determine cell density and

morphology. After pictures were taken, cells were trypsinized for cell count using Phase Hemacytometer (Hausser Scientific). Cell lysis was done for Western blot analysis.

Cell Growth Assay with ATRA treatment

Cultured cells were harvested by trypsin digestion and seeded at 300,000cells/10cm plate in the DME High glucose medium with 10% FBS for 24 hr. Cell medium was replaced with 20μM ATRA or DMSO (control) in 10% FBS DME High glucose medium (1:1000), and incubated for 1, 2, 3 and 4 days. Photos were taken from plates daily using Leica DMI6000B inverted microscope attached to DFC camera to determine cell density and morphology. Thereafter, ATRA-treated and DMSO-treated plates were trypsinized for cell count using Phase Hemacytometer (Hausser Scientific). Cells were then lysed for Western blot analysis.

Western-blot Assay

Cells were harvested and lysed in ice-cold RIPA buffer (50mM Tris-HCl, pH 7.4, 150mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with 1 tablet of Protease Inhibitor Cocktail/ 10mL RIPA buffer. Protein expressions were quantified by using the BCA Protein Assay kit (EMD Biosciences, Inc. Darmstadt, Germany). Equal amounts of protein were loaded onto 10% SDS-polyacrylamide gels and thereafter blotted to an Immobilon-P membrane (Millipore, Bedford, MA). The blots were blocked at 4°C overnight with 5% non-fat milk in TBST (500mM Tris, 54mM KCl, 2.74 M NaCl, 0.2% Tween-20) and subsequently incubated for 2 hr at room temperature with the following primary antibodies: rabbit polyclonal antibodies against human Chmp1 (prepared and purified in our lab by Dr Natalia Belogortseva) (1 μl/ml), and stbm

(prepared and purified by Dr Maiyon Park) (1 µl/ml). p53 (Cell Signaling) (1 µl/ml) and phosphorylated-p53 (pp53) ser 37 (Cell Signaling) (1 µl/ml), Bad (Cell Signaling) (1 µl/ml) and Bak(Cell Signaling) (1 µl/ml). A mouse MAb against β-actin (abCam) (0.1µl/ml) was used to confirm that the amounts of protein were equal. The blots were incubated for 1 hr with horseradish-peroxidase-conjugated secondary antibodies. Proteins were visualized by using enhanced chemiluminescent substrate detection for HRP (Amersham, Buckinghamshire, England).

RNA Extraction and Microarray Analysis

Total cellular RNA was prepared from cells treated with ATRA for 1 day and cells transfected with Chmp1 for 1 day using Tri Reagent (Sigma). In each case, cells were disrupted and homogenized in the Tri Reagent. Chloroform was mixed with the lysate, and the mixture was separated into three phases by centrifugation. The upper phase which contained the RNA was collected and precipitated with isopropanol. The RNA was used for Microarray experiment. Analysis of the results from microarray (microarray experiment and analysis were done by Dr. Goran Boskovic of the Genomic Core, Joan C. Edward School of Medicine, Marshall University) provided information on the level of gene expression from cells treated with ATRA and cells transfected with Chmp1. Differential expression of genes by microarray analysis was confirmed by Western-blot assay.

Statistical Analysis

All statistical analyses were performed with MINITAB statistical software (version 13) for the Microsoft PC. Results on cell growth are expressed as mean ±SEM. Two-sample t-test was used to compare the equality of the means of DMSO (control) and ATRA, and also between the means of CS2 (control) and Chmp1.

RESULTS

Chmp1 represses growth of pancreatic tumor cells

We investigated the effect of Chmp1 expression on the growth of cancer cells by transfecting CRL2151 cells with 5µg of Chmp1/ CS2 vector in a 10cm plate. Cells were transfected with CS2 vector and Stbm/CS2 vector to serve as control. After 1, 2, 3 or 4 days of transfection, cells were counted and observed under Leica DMI6000B inverted microscope attached to DFC camera for cell morphology.

Table 1 shows the effect of Chmp1 on the growth of CRL2151 cells. Chmp1 inhibits the growth of the cells all through the four days of transfection when compared to the control (CS2 vector). However, there was no change in the morphology of the cells (Figure 4).

Days of Transfection	Control $x10^5$	Chmp1 $x10^5$
2	7.50±0.91	3.13±0.70
3	12.50±0.91	5.00±0.72
4	28.70±1.67	7.38±0.57

Table 1: Chmp1 induces growth inhibition in Chmp1 transfected pancreatic acinar cancer cells. CRL2151 cells were seeded at 8 $x10^5$ cells/ plate in a 10cm plate in DME High glucose medium with 10% FBS. After incubation for 24hr cells were transfected (in triplicate) with Chmp1/CS2 and CS2 (control) vectors. Cells were counted after 2, 3 and 4 days of transfection and average values from six counts were obtained. The t-test analysis of the difference between the means of Chmp1 and CS2 (see appendix) indicates a marked significant difference at 95% CI on each day, as p – values << 0.005.

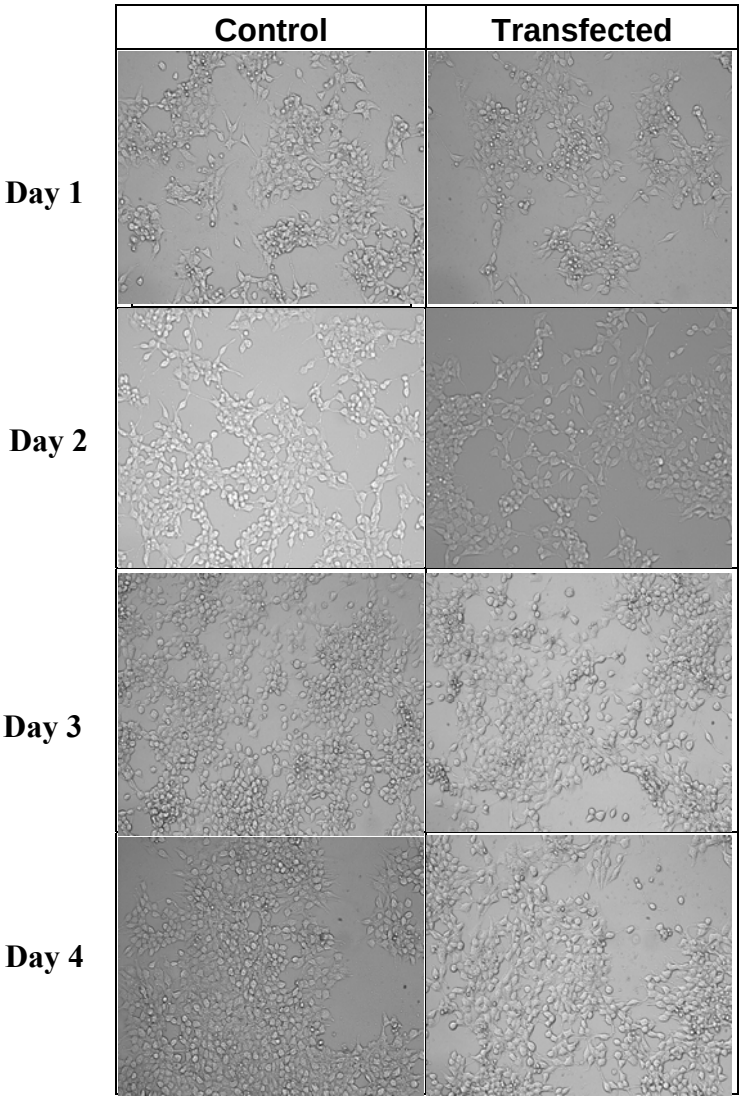


Figure 4: Chmp1 induces growth inhibition but no morphological changes in pancreatic acinar tumor cells. CRL2151 cells were transfected with Chmp1/CS2 and CS2 (control) in 10cm plates for 1, 2, 3 and 4 days. Cell density and morphology were observed using Leica DMI6000B inverted microscope attached to DFC camera. Growth was inhibited in Chmp1 transfected cells (right) compared to CS2 transfected cells (control) (left) but no morphological changes at day 1 - 4.

Retinoic acid treatment induces the expression of Chmp1 and p53

To investigate the effect retinoic acid has on the growth of acinar carcinoma cells, we counted CRL2151 cells and observed the cells under Leica DMI6000B inverted microscope attached to DFC camera for cell morphology after treatment with ATRA. We also performed Western blot analysis using antibodies which recognize Chmp1 (25kDa), p53 (53 kDa) and Stbm (75 kDa) to investigate the expression levels of these proteins in the treated cells.

The growth curve of retinoic acid-treated cells showed no decrease in growth due to retinoic acid treatment (Figure 5).

Observation of these cells under microscope did not show morphological differences between the retinoic acid treated cells and the control treated cells (Figure 6). This is contrary to the earlier results obtained in our lab from similar treatment on adinocarcinoma PanC-1 cells (Figure I). In this case, less growth was observed in retinoic acid treated panC-1 cells. Observation of these PanC-1 cells under microscope after four days of retinoic acid treatment showed less cell growth as well as morphological changes.

Treatment of CRL2151 cells with retinoic acid however showed over-expression of Chmp1 (4.5-fold) at day 1 compared to its expression in DMSO treated cells on the same day (Figure 7). Chmp1 over-expression was accompanied by up-regulation of p53 (3.7-fold) at day 1. Similar expression of Chmp1 and p53 was observed in Panc-1 at day 2 (Figures II & III). Even though stbm was over-expressed at day 4, there was no accompanying up-regulation of p53 (Figure 7).

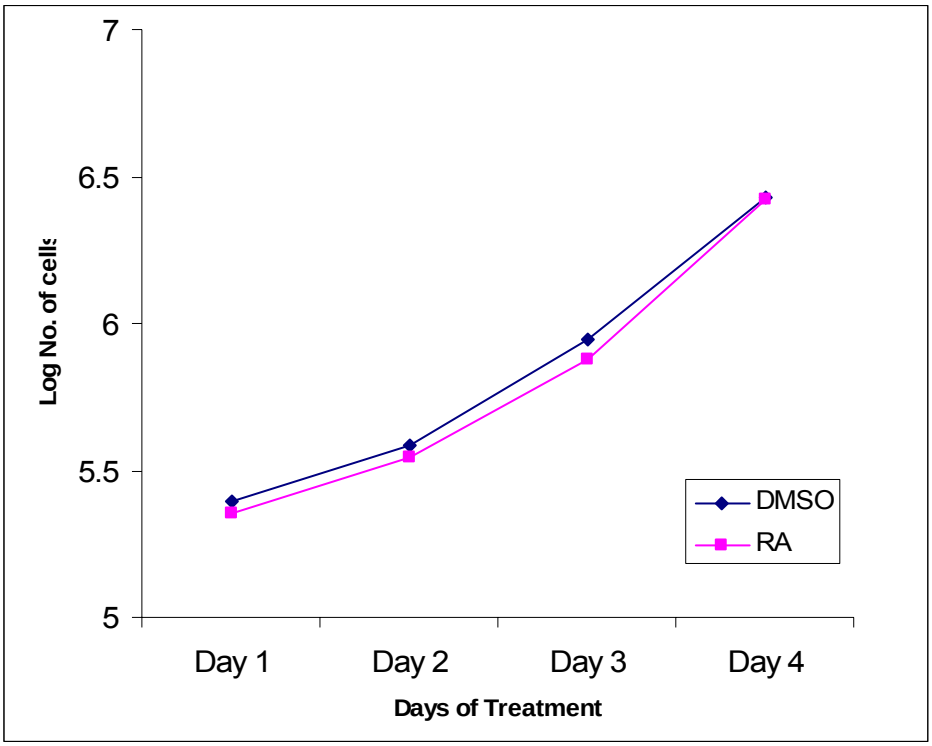


Figure 5: Retinoic acid does not inhibit growth of pancreatic acinar tumor cells. CRL2151 cells were seeded at 3 x10^5 cells/ plate in a 10cm plate in DME High glucose medium with 10% FBS. After incubation for 24hr cells were treated with 20μM ATRA and DMSO (control) (in triplicate) for 1, 2, 3, and 4 days. Cells were counted after each period of treatment. Data represent the mean ± standard errors of the mean (SE) from six counts. The t-test analysis of the difference between the means of DMSO and RA (see appendix) indicates no significant difference at 95% CI on each day.

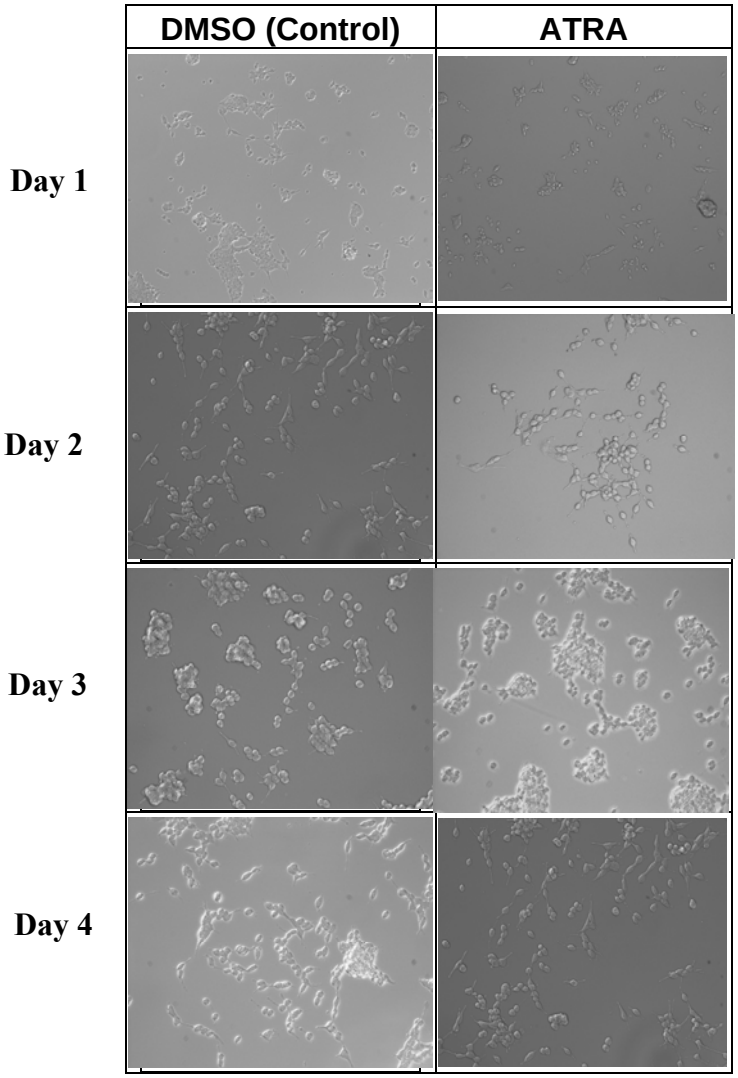
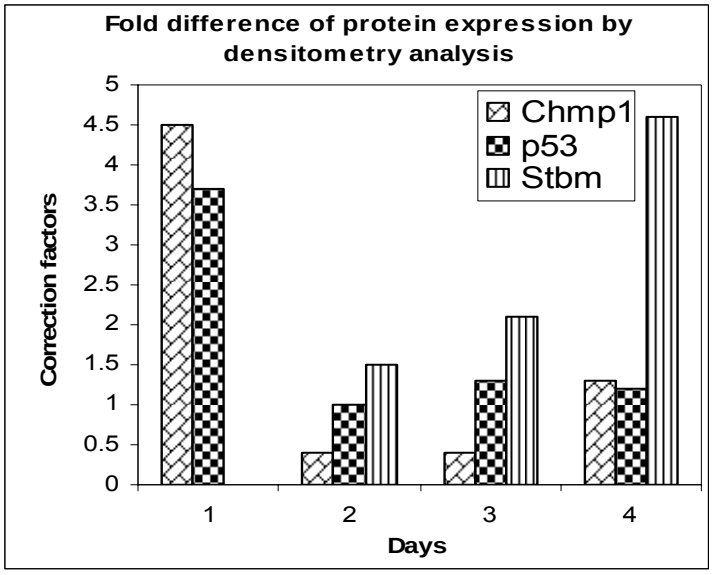


Figure 6: ATRA does not induce growth inhibition or morphology change in ATRA-treated pancreatic acinar tumor cells. CRL2151 cells were seeded at 3 x10^5 cells/ plate in a 10cm plate in DME High glucose medium with 10% FBS. After incubation for 24hr cells were treated with 20μM ATRA and DMSO (control) (in triplicate) for 1, 2, 3, and 4 days. Cell density and morphology were observed using Leica DMI6000B inverted microscope attached to DFC camera. There was no growth inhibition in ATRA-treated cells (right) compared to DMSO treated cells (control) (left).No morphological changes at day 1 - 4.



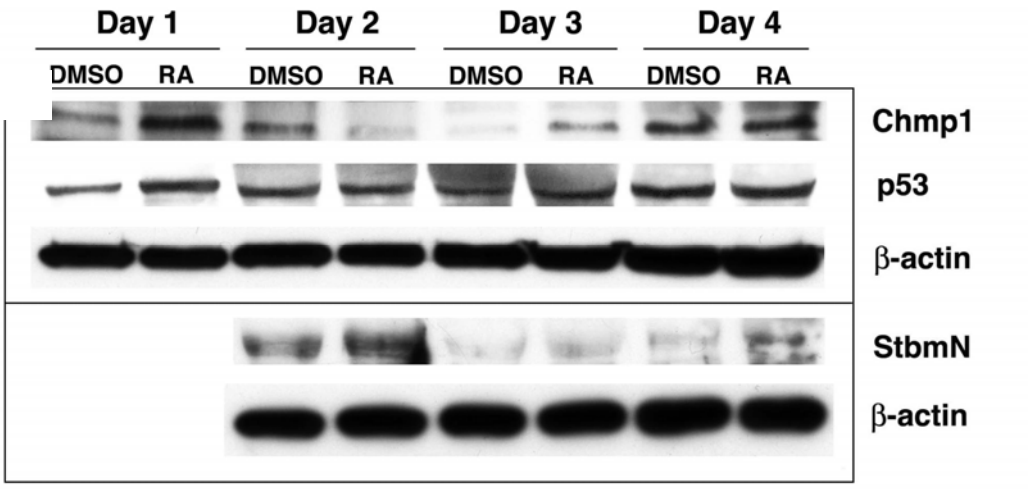


Figure 7: CRL 2151 cells were treated with ATRA(20μM) and DMSO(control) in a 10cm plate of DME High glucose medium containing 10% FBS for 1, 2, 3 and 4 days. Cells were lysed, 5 micrograms of protein were used for Western blot and immunodetection was done for Chmp1, p53 and Stbm. Graph shows the expressions of Chmp1, p53 and Stbm corrected for β-actin and DMSO from densitometry analysis.

Chmp1 induces the expression of p53

CRL2151 cells were transfected with 5µg of Chmp1/ CS2 vector in a 10cm plate. Cells were also transfected with CS2 vector and Stbm/CS2 vector to serve as control. Western blot analysis was carried out using antibodies which recognize Chmp1 (25kDa), Stbm (73 kDa), p53 (53 kDa) and pp53 (ser 37, 53 kDa). Results showed that the expression of Chmp1 was temporarily increased (3.7-fold) at day 1 in cells transfected with Chmp1/CS2 vector (Figure 8). Chmp1 over-expression was accompanied by p53 up regulation (3.3-fold) at day 1. This trend was followed by pp53 (ser 37) up-regulation (3.2-fold) at day 1 too. The low expression of Chmp1 at day 2 is interesting, and may suggest that the expression of Chmp1 is repressed with time. Results from PanC-1 cell line investigated in our lab did not show Chmp1 repression with time. Even though Strabismus was up-regulated at day 1 and day 2 in Stbm-transfected cells, there was no effect on p53 and pp53 protein level. This result demonstrates that p53 expression is specific to Chmp1 expression.

Protein expression in CRL2151 cells by microarray assay

To confirm that Chmp1 functions through retinoic acid by activating p53, microarray analysis was performed on cells transfected with Chmp1 for 1 day and cells treated with retinoic acid for 1 day. If Chmp1 functions through retinoic acid signaling we expect the same genes to be up or down regulated when cells are treated with retinoic acid or transfected with Chmp1. Table 2 shows few of same set of genes that were up or down regulated. Of particular interest to us is the up-regulation of Bad and Bak which promote apoptosis and the down regulation of cell cycle division associated proteins. Western lot

assay confirmed the expression of Bad and Bak genes in cells transfected with Chmp1/CS2 vector at day 2 and day 1 respectively (Figure 9).

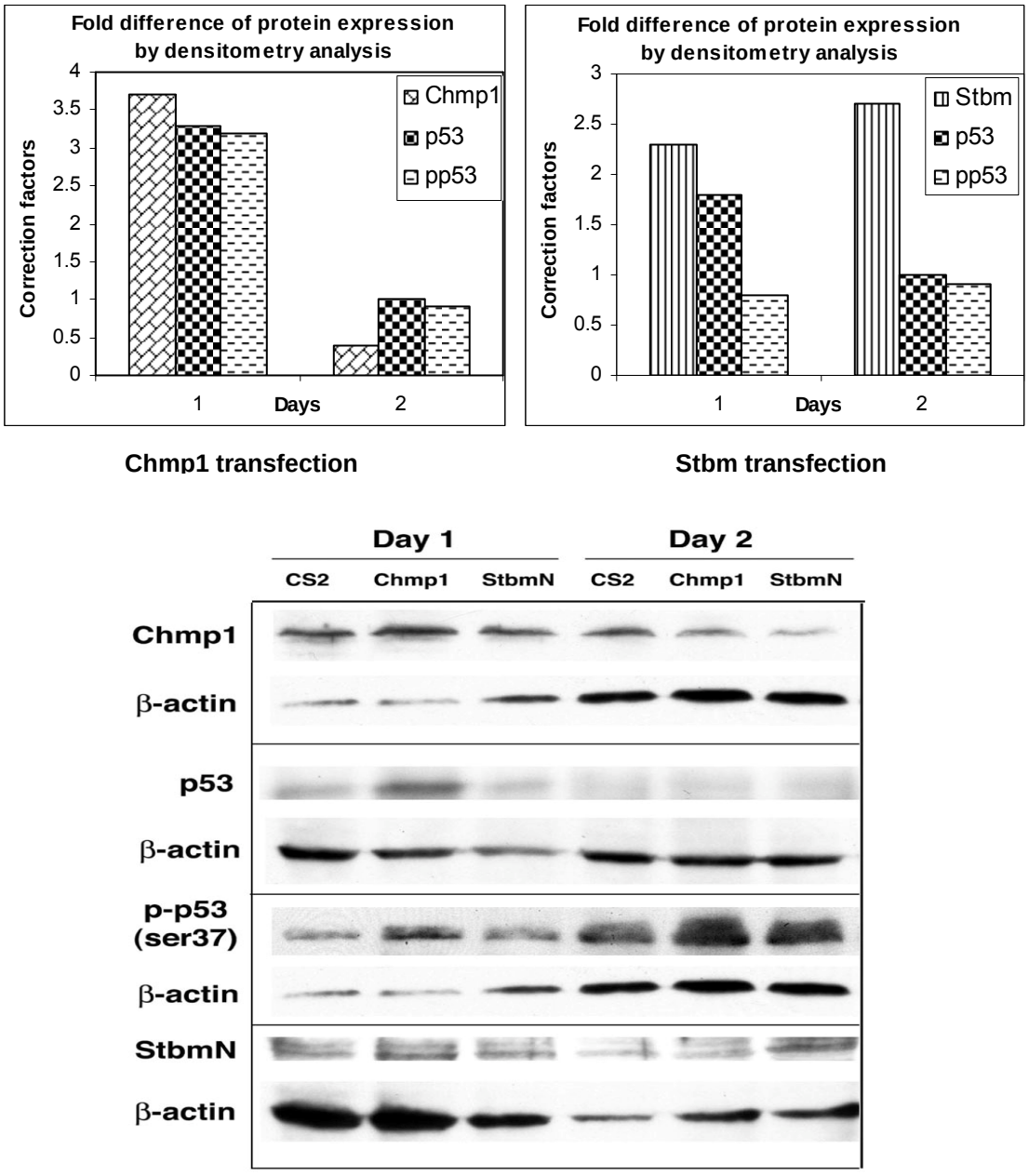
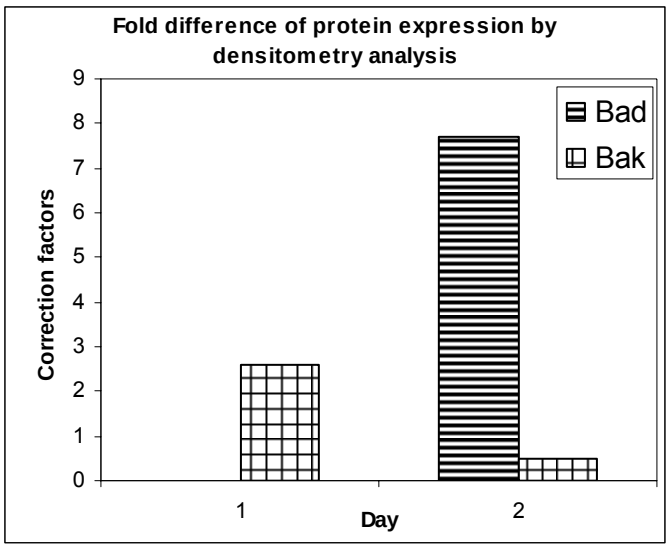


Figure 8: CRL 2151 cells were transfected with Chmp1/CS2, Stbm/CS2 and CS2 (control) vectors in a 10cm plate of DME High glucose medium containing 10% FBS for 1 and 2 days. Cells were lysed for Western blot with immunodetection for Chmp1, p53, pp53 (ser37) and Stbm. Graphs show the expressions of Chmp1, p53, pp53 and Stbm corrected for β-actin and CS2 from densitometry analysis.

UP Regulated				Down Regulated			
Official Symbol	Gene ID	Name	Function (Expression Fold)	Official Symbol	Gene ID	Name	Function (Expression Fold)
Bak1	12018	Bcl2-antagonist	Proapoptotic (2)	Sfrs1	110809	Splicing factor, arginine/serine-rich1	Splicing of pre-mRNA (2)
Bad	12015	Bcl-associated death promoter	Proapoptotic (2)	Cdca7	66953	Cell division cycle associated 7	Overexpressed in human tumors (2.5)
Slc9a8	77031	Solute carrier family	Control of intracellular pH (2)	Rpl22	19934	Ribosomal protein L22	Regulation of protein synthesis (3)
Prrx1	18933	Paired related homeobox1	Cranial development (2)	Vbp1	22327	Von Hippel-Lindau binding protein 1	Tumor suppressor gene (2)
Barx2	12023	BarH-like homeobox2	Cellular differentiation (34)	Oazin or Oazi	54375	Ornithine decarboxylase antizyme inhibitor	Tumor inducer, cell growth and division (2)
				Hnrpab	15384	Heterogenous nuclear ribonucleo protein A/B	RNA splicing, transport (3)

Table 2: Microarray results of total mRNA from CRL 2151 cells transfected with Chmp1 for I day and cells treated with ATRA for I day. After treatment with retinoic acid or transfection with Chmp1/CS2, RNA was made using the Tri reagent. cDNA was generated and hybridized to agilent whole genome mouse 44k array for analysis. Genes in the table are expressed in transfected and treated cells.



Chmp1 transfection

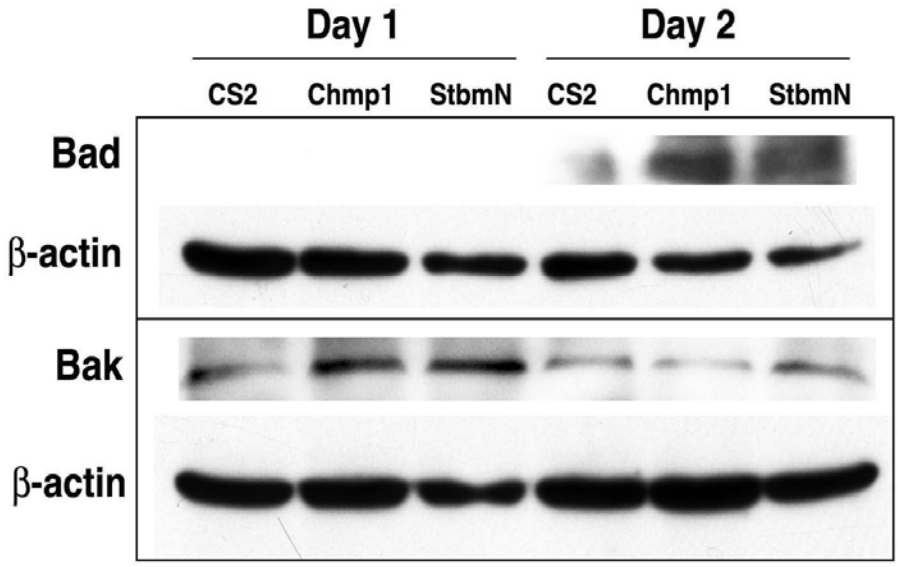


Figure 9: Chmp1 induces the expression of Bcl-2 family. CRL 2151 cells were transfected with Chmp1/CS2, Stbm/CS2 and CS2 (control) vectors in a 10cm plate of DME High glucose medium contining10% FBS for 1 and 2 days. Cells were lysed for Western blot with immunodetection for Bad and Bak. Graph shows the expressions of Bad and Bak corrected for β-actin and CS2 from densitometry analysis.

DISCUSSION

The present study has led to a new finding about Chmp1 function in the suppression of pancreatic tumor through the retinoic acid pathway. Chmp1 is thought to play a role in the regulation of gene expression when located in the nucleus [2-4]. It was also observed that when zebrafish embryos were microinjected with Chmp1, it induced hyperplasia. On the basis of this observation and our results from the human cancer profiling array for Chmp1, it is possible that Chmp1 may be involved in suppressing the growth of tumor cells. We therefore hypothesized that Chmp1 may play a role in inhibiting cell growth through retinoic acid signaling by activating p53. Protein p53 is reported to be expressed and regulate cell growth and apoptosis during cancer cell growth inhibition [35]. In this study we have shown that exogenous Chmp1 indeed resulted in the inhibition of growth (Table 1) however, without any morphological change of cells (Figure 4).

Retinoids are therapeutically used in the treatment of some cancers [31]. They are reported to cause a decrease in the number of cells in pancreatic cancer [9, 16-17]. Results from our study did not show decrease in the growth of cells due to retinoic acid treatment. This result is not surprising. A similar result was reported by Rosewicz et al (1995), in which pancreatic acinar tumor cells did not show growth inhibition after treatment with retinoids. Furthermore, Fujimoto et al (1999) reported that some pancreatic tumor cell lines did not show growth inhibition when treated with retinoids.

It is not known if Chmp1 expression is induced by retinoic acid treatment, nor what function it may carryout if expressed. In this study, our result was able to show that retinoic acid treatment induced the over-expression of Chmp1. This over-expression of Chmp1 was accompanied by the up regulation of p53. Our further investigation showed expression of Chmp1, p53 and pp53 (ser 37) to be up-regulated in cells transfected with Chmp1 (Figure 8), accompanied by growth inhibition. Taken together, these results suggest that Chmp1 inhibits cell growth by inducing the activation of p53 through RA signaling pathway.

Strabismus, a binding partner of Chmp1 from the yeast 2-hybrid screening carried out in our lab, was up-regulated at day 1 and day 2. However, it did not show effect on p53 and pp53 protein level. This observation shows that even though Strabismus functions in vertebrate extension movement, the induction of hyperplasia and tumor suppression is rather specific to Chmp1 expression.

Microarray analysis of cells treated with ATRA and cells transfected with Chmp1 showed up-regulation of Bad and Bak (pro-apoptosis proteins), in addition to other proteins up-regulated. Cell division cycle associated protein 7 (Cdca7) was among proteins down-regulated. Cdca7 is frequently over-expressed in human cancers[74]. The down-regulation of Cdca7 and up-regulation of Bad and Bak in our study further supports the suggestion that Chmp1 is involved in growth inhibition of CRL2151 cells, consequently functioning in suppressing cancer growth.

In summary, the results from this study have shown that Chmp1 induces growth inhibition in pancreatic tumor. Its expression is induced by retinoic acid in retinoic acid treated pancreatic acinar tumor cells. Microarray analysis showed similar genes to be

up or down regulated in cells treated with retinoic acid and cells transfected with Chmp1.

ACKNOWLEDGEMENT

This study was supported by NIH COBRE Grant to Dr Maiyon Park.

CHAPTER 4

CONCLUSIONS

1. Chmp1 was found to induce growth inhibition but no morphological changes when CRL 2151 cells were transfected with Chmp1.

2. Over-expression of Chmp1 was induced in ATRA-treated cells accompanied by the up-regulation of p53.

3. Chmp1 induced the up-regulation of p53 and also induced its stability by the up-regulation of pp53-ser 37 in Chmp1-transfected CRL 2151 cells. Strabismus did not show effect on p53 and pp53-ser 37 protein levels.

4. Similar proteins were up-regulated or down-regulated when CRL2151 cells were treated with ATRA and transfected with Chmp1. Apoptotic agonists, Bad and Bak, were among proteins up-regulated.

FUTURE DIRECTIONS

Future direction on this study will focus on confirming other up and down regulated genes from microarray analysis of cells transfected with Chmp1 and cells treated with retinoic acid. This will include confirming Bad and Bak in RA treated cells.

Since our results showed that p53 and pp53 expression accompanied the expression of Chmp1, investigating upstream proteins in p53 pathway (e.g. DNA-PK, ATM) will provide information whether activated p53 induces growth arrest or apoptosis or both during cell growth inhibition. This will be carried out by Western blot analysis of lysate from CRL2151 cells transfected with Chmp1. Test for apoptosis will be done using e.g. Tunel assay, Serine assay or Caspase detection assay. Also Chmp1 transfection efficiency using the GFP cotransfection method will be determined.

We will further investigate the functions of Chmp1 by siRNA silencing of Chmp1 gene in CRL2151 cells. This will include studying the expression levels of p53 and pp53 after Chmp1 knock down in the cells.

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APPENDIX

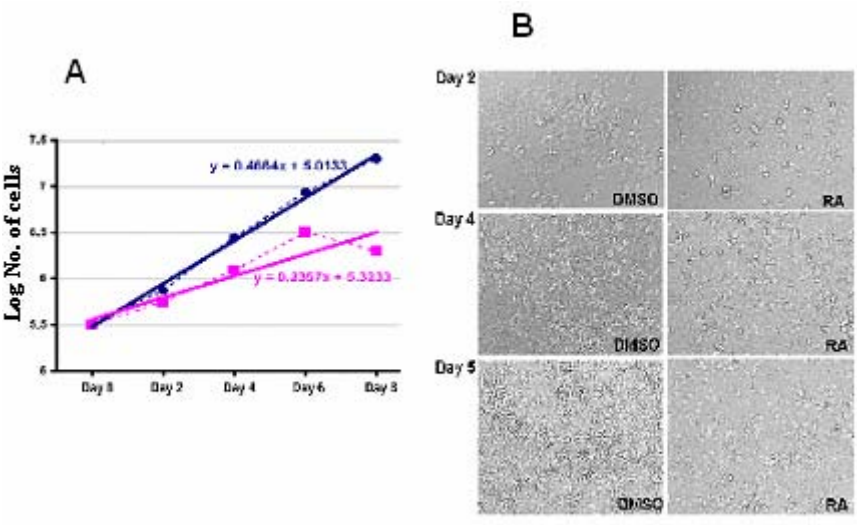


Figure I: Treatment of panc-1 cells with ATRA (10μM) inhibits growth and results in morphology changes. A- growth curve of panc-1 treated with ATRA. B- changes in cell morphology observed under Leica inverted microscope attached to DFC camera. (Courtesy of Brandon Orr).

---■---■---■--- RA; ---♦---♦---♦--- DMSO

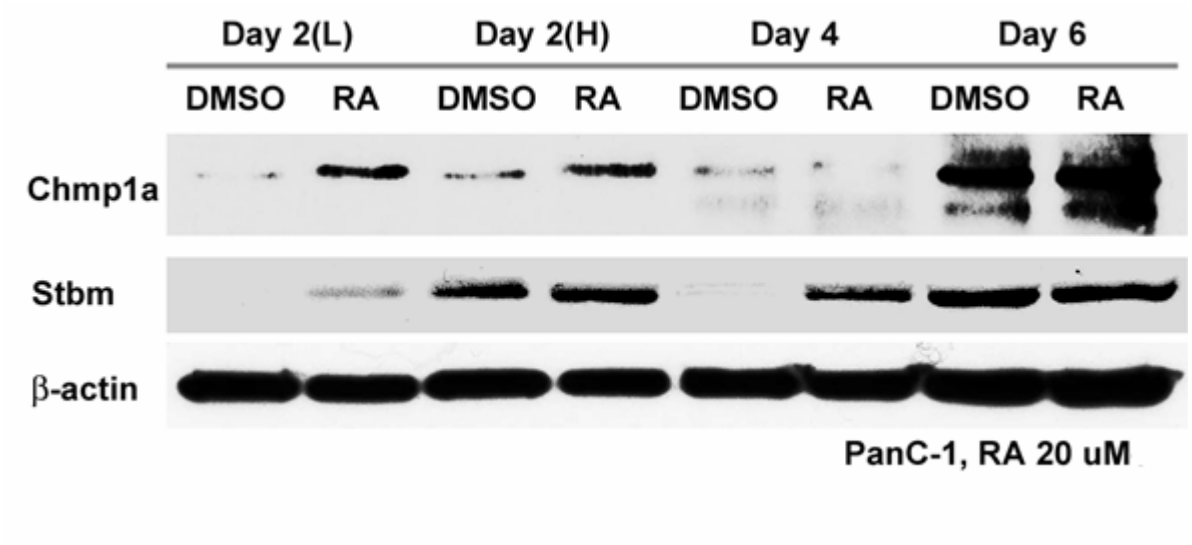


Figure II: Western blot of panc-1 cells after treatment with 20μM ATRA. L- Low confluency. H- High confluency (Courtesy of Brandon Orr).

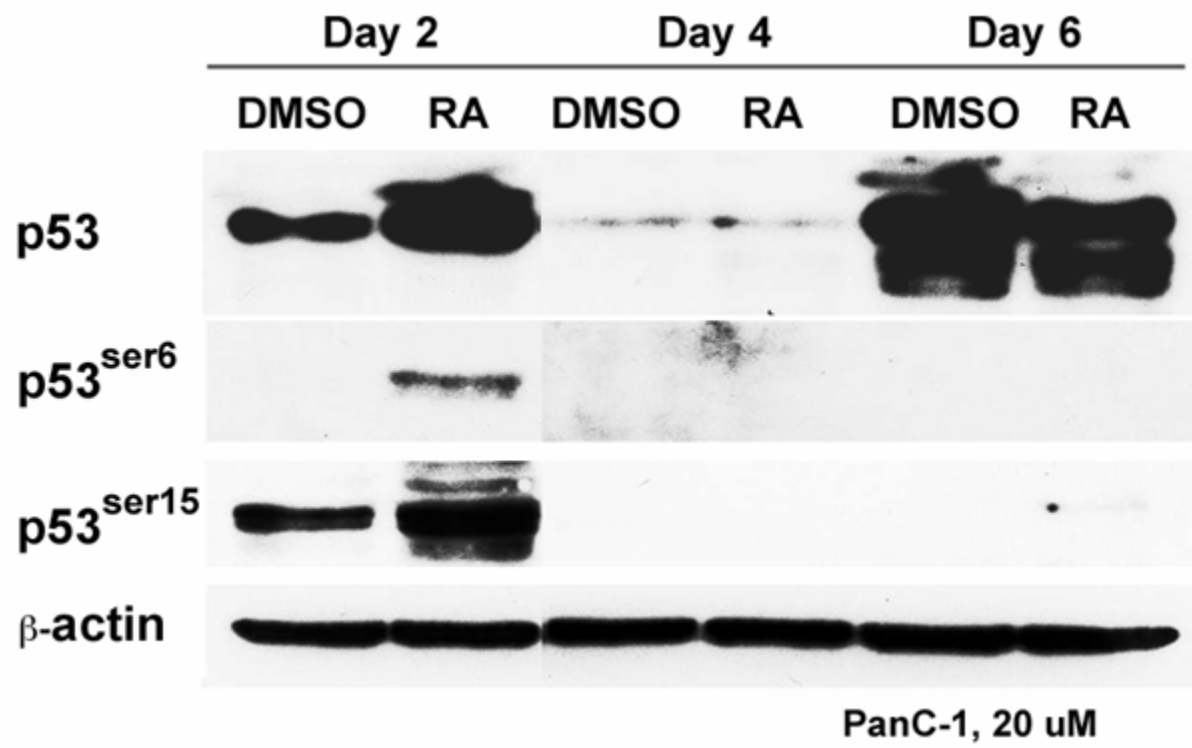


Figure III: Western blot of panc-1 cells after treatment with 20μM ATRA (Courtesy of Brandon Orr).

Raw data

Raw data table for ATRA treated growth curve.

Day	Day1	Day1	Day2	Day2	Day3	Day3	Day4	Day4
Treatment	DMSO	RA	DMSO	RA	DMSO	RA	DMSO	RA
Raw Data	2.50E+05	1.25E+05	5.00E+05	5.00E+05	7.00E+05	6.25E+05	2.00E+06	2.50E+06
	2.20E+05	3.50E+05	2.75E+05	2.50E+05	1.00E+06	6.25E+05	2.50E+06	2.75E+06
	3.75E+05	1.25E+05	3.75E+05	3.75E+05	5.00E+05	8.75E+05	2.63E+06	2.00E+06
	2.50E+05	2.50E+05	6.75E+05	6.23E+05	8.75E+05	1.25E+06	2.25E+06	2.63E+06
	2.50E+05	2.50E+05	1.25E+05	1.25E+05	8.75E+05	6.25E+05	3.13E+06	3.00E+06
	1.25E+05	2.50E+05	3.75E+05	2.50E+05	1.38E+06	5.00E+05	3.63E+06	2.88E+06
Mean	2.45E+05	2.25E+05	3.88E+05	3.54E+05	8.88E+05	7.50E+05	2.69E+06	2.63E+06
Standard Error of the mean	3.27E+04	3.54E+04	7.69E+04	7.49E+04	1.21E+05	1.12E+05	2.43E+05	1.44E+05
Standard Deviation	8.00E+04	8.66E+04	1.88E+05	1.83E+05	2.95E+05	2.74E+05	5.96E+05	3.54E+05
Count	6	6	6	6	6	6	6	6

Two-Sample T-Test for DMSO and RA (Day1)

	N	Mean	StDev	SE Mean
DMSO	6	245000	80000	32660
RA	6	225000	86603	35355

Difference = μ DMSO - μ RA
Estimate for difference: 20000
95% CI for difference: (-88882, 128882)
T-Test of difference = 0 (vs. not =): T-Value = 0.42 P-Value = 0.687 DF = 10

Two-Sample T-Test for DMSO and RA (Day2)

	N	Mean	StDev	SE Mean
DMSO	6	387500	188249	76852
RA	6	353833	183407	74876

Difference = μ DMSO - μ RA
Estimate for difference: 33667
95% CI for difference: (-209056, 276389)
T-Test of difference = 0 (vs. not =): T-Value = 0.31 P-Value = 0.761 DF = 10

Two-Sample T-Test for DMSO and RA (Day3)

	N	Mean	StDev	SE Mean
DMSO	6	887500	295275	120546
RA	6	750000	273861	111803

Difference = μ DMSO - μ RA
Estimate for difference: 137500
95% CI for difference: (-234425, 509425)
T-Test of difference = 0 (vs. not =): T-Value = 0.84 P-Value = 0.425 DF = 10

Two-Sample T-Test for DMSO and RA (Day4)

	N	Mean	StDev	SE Mean
DMSO	6	2687500	595556	243135
RA	6	2625000	353553	144338

Difference = μ DMSO - μ RA
Estimate for difference: 62500
95% CI for difference: (-589524, 714524)
T-Test of difference = 0 (vs. not =): T-Value = 0.22 P-Value = 0.831 DF = 10

Raw data

Raw data table for Chmp1 transfected growth curve.

Day	Day2	Day2	Day3	Day3	Day4	Day4
Vector	CS2	Chmp1	CS2	Chmp1	CS2	Chmp1
Raw data	6.25E+05	2.50E+05	1.38E+06	3.75E+05	3.50E+06	8.75E+05
	3.75E+05	2.50E+05	1.13E+06	2.50E+05	2.88E+06	7.50E+05
	7.50E+05	3.75E+05	8.75E+05	5.00E+05	2.88E+06	7.50E+05
	8.75E+05	6.25E+05	1.25E+06	6.25E+05	2.38E+06	5.00E+05
	8.75E+05	1.25E+05	1.38E+06	7.50E+05	3.10E+06	6.75E+05
	1.00E+06	2.50E+05	1.50E+06	5.00E+05	2.50E+06	8.75E+05
Mean	7.50E+05	3.13E+05	1.25E+06	5.00E+05	2.87E+06	7.38E+05
Standard Error of the mean	9.13E+04	7.03E+04	9.13E+04	7.22E+04	1.67E+05	5.73E+04
Standard Deviation	2.24E+05	1.72E+05	2.24E+05	1.77E+05	4.08E+05	1.40E+05
Count	6	6	6	6	6	6

Two-Sample T-Test for CS2 and Chmp1 (Day2)

	N	Mean	StDev	SE Mean
CS2	6	750000	223607	91287
Chmp1	6	312500	172301	70341

Difference = μ CS2 - μ Chmp1
Estimate for difference: 437500
95% CI for difference: (176799, 698201)
T-Test of difference = 0 (vs. not =): T-Value = 3.80 P-Value = 0.004 DF = 10

Two-Sample T-Test for CS2 and Chmp1 (Day3)

	N	Mean	StDev	SE Mean
CS2	6	1252500	224182	91522
Chmp1	6	500000	176777	72169

Difference = μ CS2 - μ Chmp1
Estimate for difference: 752500
95% CI for difference: (488839, 1016161)
T-Test of difference = 0 (vs. not =): T-Value = 6.46 P-Value = 0.000 DF = 10

Two-Sample T-Test for CS2 and Chmp1 (Day4)

	N	Mean	StDev	SE Mean
CS2	6	2873333	406678	166025
Chmp1	6	737500	140312	57282

Difference = μ CS2 - μ Chmp1
Estimate for difference: 2135833
95% CI for difference: (1705965, 2565702)
T-Test of difference = 0 (vs. not =): T-Value = 12.16 P-Value = 0.000 DF = 10

Film properties report for Chmp1 (transfected)

Cell type: CRL 2151 Protein quantity: 5µg

Gel type: 10% Tris-HCL SDSPAGE Electrophoresis Voltage: 100V

Transfer Voltage: 100V Duration: 45min

Primary Antibody: Chmp1 Dilution: 1: 1000 Medium: 5% milk in TBS-T

Secondary Antibody: Anti Rabbit Dilution: 1: 1000 Medium: 5% milk in TBS-T

Molecular weight: 25 kDa

Lane 1: ProSieve color protein marker 5µl

Lane 2: CS2 Day1

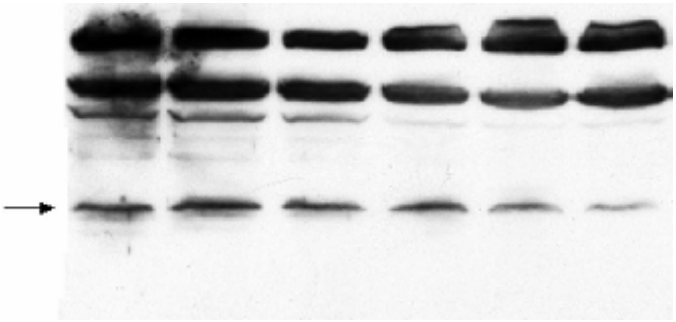
Lane 3: Chmp1 Day1

Lane 4: Stbm Day1

Lane 5: CS2 Day2

Lane 6: Chmp1 Day2

Lane 7: Stbm Day2



Film properties report for Chmp1-βactin (transfected)

Cell type: CRL 2151 Protein quantity: 5µg

Gel type: 10% Tris-HCL SDSPAGE Electrophoresis Voltage: 100V

Transfer Voltage: 100V Duration: 45min

Primary Antibody: β-actin (abCam) Dilution: 1: 10000 Medium: 5% milk in TBS-T

Secondary Antibody: Anti Mouse Dilution: 1: 10000 Medium: 5% milk in TBS-T

Molecular weight: 42 kDa

Lane 1: ProSieve color protein marker 5µl

Lane 2: CS2 Day1

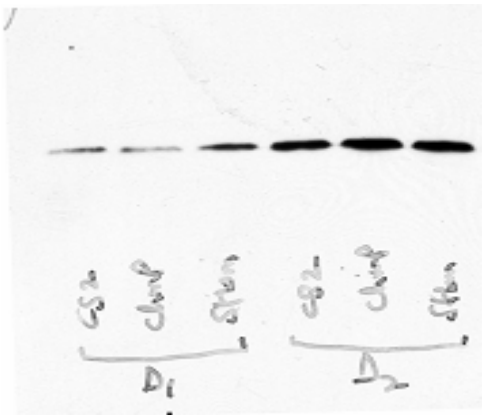
Lane 3: Chmp1 Day1

Lane 4: Stbm Day1

Lane 5: CS2 Day2

Lane 6: Chmp1 Day2

Lane 7: Stbm Day2



Raw data table produced from spot densitometry of Western blot films.

Chmp1-Transfected

Adj. Vol. INT			
Trf-Chmp	Trf-Chmpactin	Trf-Chmp(ratio)	Trf-Chmp(corr)
2149.88	1409.27	1.525527401	1
5611.76	982.58	5.711249975	3.743787212
2905.17	2977.67	0.975652104	0.639550691
2925.06	5940.36	0.492404501	1
1619.69	7128.46	0.227214574	0.461438864
735.05	6746.6	0.108951175	0.221263565

Film properties report for Stbm (transfected)

Cell type: CRL 2151 Protein quantity: 5µg

Gel type: 10% Tris-HCL SDSPAGE Electrophoresis Voltage: 100V

Transfer Voltage: 100V Duration: 45min

Primary Antibody: StbmN Dilution: 1: 1000 Medium: 5% milk in TBS-T

Secondary Antibody: Anti Rabbit Dilution: 1: 1000 Medium: 5% milk in TBS-T

Molecular weight: 75 kDa

Lane 1: ProSieve color protein marker 5µl

Lane 2: CS2 Day1

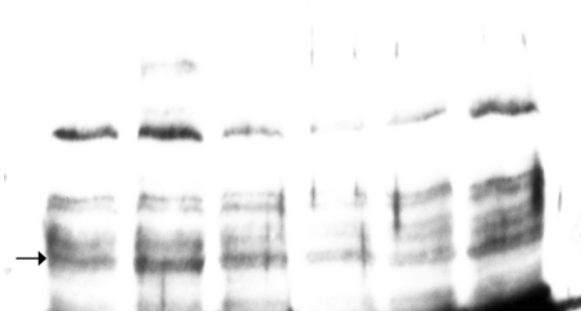
Lane 3: Chmp1 Day1

Lane 4: Stbm Day1

Lane 5: CS2 Day2

Lane 6: Chmp1 Day2

Lane 7: Stbm Day2



Film properties report for Stbm- βactin (transfected)

Cell type: CRL 2151 Protein quantity: 5µg

Gel type: 10% Tris-HCL SDSPAGE Electrophoresis Voltage: 100V

Transfer Voltage: 100V Duration: 45min

Primary Antibody: β-actin (abCam) Dilution: 1: 10000 Medium: 5% milk in TBS-T

Secondary Antibody: Anti Mouse Dilution: 1: 10000 Medium: 5% milk in TBS-T

Molecular weight: 42 kDa

Lane 1: ProSieve color protein marker 5µl

Lane 2: CS2 Day1

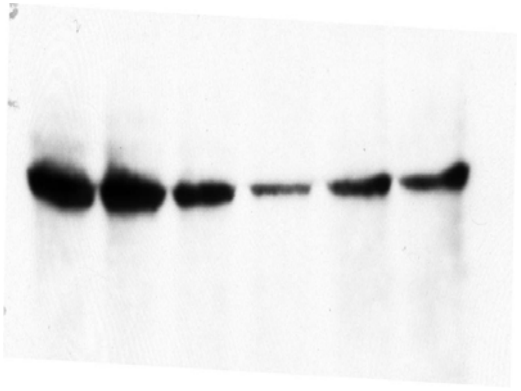
Lane 3: Chmp1 Day1

Lane 4: Stbm Day1

Lane 5: CS2 Day2

Lane 6: Chmp1 Day2

Lane 7: Stbm Day2



Raw data table produced from spot densitometry of Western blot films.

Strabismus-Transfected

Adj. Vol. INT			
Trf-Stbm	**Trf-Stbmactin**	**Trf-Stbm(ratio)**	**Trf-Stbm(corr)**
1337.14	15658.81	0.09	1.0
1928.95	14340.55	0.13	1.6
1531.08	7863.4	0.19	2.3
666.47	2578.45	0.26	1.0
917.46	6110.94	0.15	0.6
4729.11	6682.03	0.71	2.7

Film properties report for p53 (transfected)

Cell type: CRL 2151 Protein quantity: 5µg

Gel type: 10% Tris-HCL SDSPAGE Electrophoresis Voltage: 100V

Transfer Voltage: 100V Duration: 45min

Primary Antibody: p53 (Cell Signaling) Dilution: 1: 1000 Medium: 5% milk in TBS-T

Secondary Antibody: Anti Rabbit Dilution: 1: 1000 Medium: 5% milk in TBS-T

Molecular weight: 53 kDa

Lane 1: ProSieve color protein marker 5µl

Lane 2: CS2 Day1

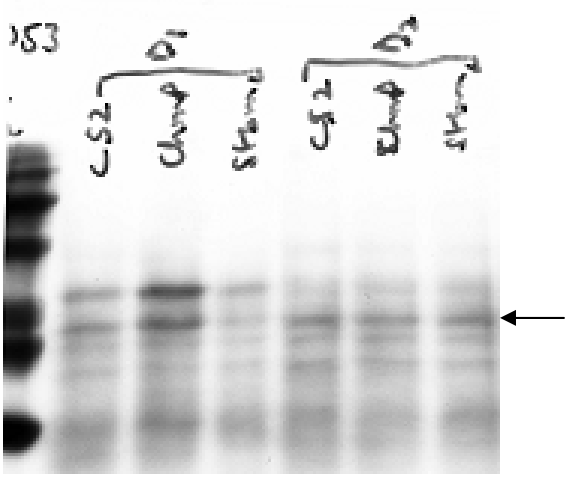
Lane 3: Chmp1 Day1

Lane 4: Stbm Day1

Lane 5: CS2 Day2

Lane 6: Chmp1 Day2

Lane 7: Stbm Day2



Film properties report for p53- βactin (transfected)

Cell type: CRL 2151 Protein quantity: 5µg

Gel type: 10% Tris-HCL SDSPAGE Electrophoresis Voltage: 100V

Transfer Voltage: 100V Duration: 45min

Primary Antibody: β-actin (abCam) Dilution: 1: 10000 Medium: 5% milk in TBS-T

Secondary Antibody: Anti Mouse Dilution: 1: 10000 Medium: 5% milk in TBS-T

Molecular weight: 42 kDa

Lane 1: ProSieve color protein marker 5µl

Lane 2: CS2 Day1

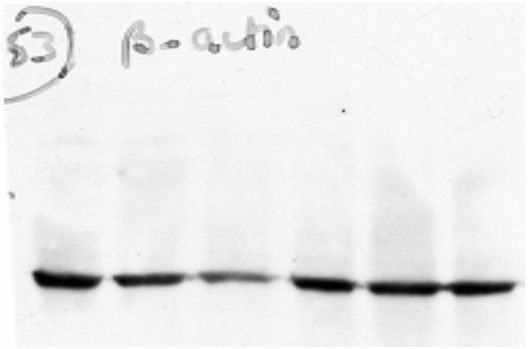
Lane 3: Chmp1 Day1

Lane 4: Stbm Day1

Lane 5: CS2 Day2

Lane 6: Chmp1 Day2

Lane 7: Stbm Day2



Raw data table produced from spot densitometry of Western blot films.

P53-Transfected

Adj. Vol. INT			
Trf-p53	**Trf-p53actin**	**Trf-p53(ratio)**	**Trf-p53(corr)**
4173.25	7083.78	0.59	1.00
8404.02	4309.6	1.95	3.31
3018.73	2802.82	1.07	1.82
2939.58	4804.18	0.61	1.00
2916.19	5010.87	0.58	0.95
3323.25	5365.64	0.62	1.01

Film properties report for phosphor p53- ser 37 (transfected)

Cell type: CRL 2151 Protein quantity: 5µg

Gel type: 10% Tris-HCL SDSPAGE Electrophoresis Voltage: 100V

Transfer Voltage: 100V Duration: 45min

Primary Antibody: pp53-ser 37 (Cell Signaling) Dilution: 1: 1000 Medium: 5% milk in TBS-T

Secondary Antibody: Anti Rabbit Dilution: 1: 1000 Medium: 5% milk in TBS-T

Molecular weight: 53 kDa

Lane 1: ProSieve color protein marker 5µl

Lane 2: CS2 Day1

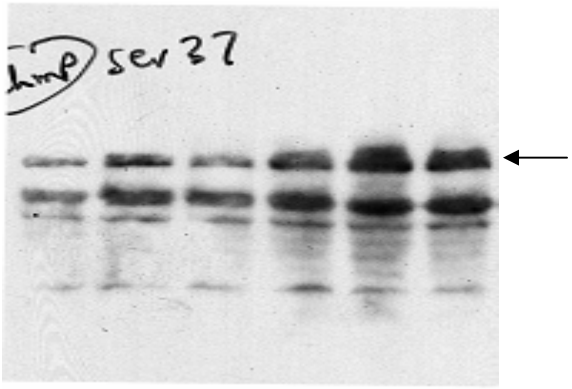
Lane 3: Chmp1 Day1

Lane 4: Stbm Day1

Lane 5: CS2 Day2

Lane 6: Chmp1 Day2

Lane 7: Stbm Day2



Film properties report for pp53 ser37-βactin (transfected)

Cell type: CRL 2151 Protein quantity: 5µg

Gel type: 10% Tris-HCL SDSPAGE Electrophoresis Voltage: 100V

Transfer Voltage: 100V Duration: 45min

Primary Antibody: β-actin (abCam) Dilution: 1: 10000 Medium: 5% milk in TBS-T

Secondary Antibody: Anti Mouse Dilution: 1: 10000 Medium: 5% milk in TBS-T

Molecular weight: 42 kDa

Lane 1: ProSieve color protein marker 5µl

Lane 2: CS2 Day1

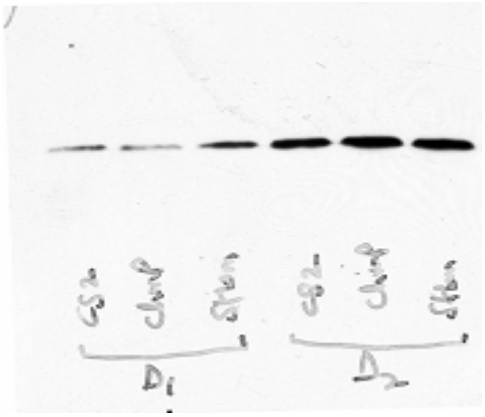
Lane 3: Chmp1 Day1

Lane 4: Stbm Day1

Lane 5: CS2 Day2

Lane 6: Chmp1 Day2

Lane 7: Stbm Day2



Raw data table produced from spot densitometry of Western blot films.

Pp53 (ser 37)-Transfected

Adj. Vol. INT			
Trf-ser37	**Trf-ser37-actin**	**Trf-ser37(ratio)**	**Trf-ser37(corr)**
4713.86	1409.27	3.344894875	1
10634.81	982.58	10.82335281	3.235782651
7871.56	2977.67	2.643530008	0.790317815
11386.88	5940.36	1.916866991	1
13476.64	7128.46	1.890540173	0.986265704
12789.99	6746.6	1.895768239	0.988993106

Film properties report for Chmp1 (ATRA treated)

Cell type: CRL 2151 Protein quantity: 5µg

Gel type: 10% Tris-HCL SDSPAGE Electrophoresis Voltage: 100V

Transfer Voltage: 100V Duration: 45min

Primary Antibody: Chmp1 Dilution: 1: 1000 Medium: 5% milk in TBS-T

Secondary Antibody: Anti Rabbit Dilution: 1: 1000 Medium: 5% milk in TBS-T

Molecular weight: 25 kDa

Lane 1: ProSieve color protein marker 5µl

Lane 2: RA Day1

Lane 3: DMSO Day1

Lane 4: RA Day2

Lane 5: DMSO Day2

Lane 6: RA Day3

Lane 7: DMSO Day3

Lane 8: RA Day4

Lane 9: DMSO Day4

Film properties report for Chmp1-βactin (ATRA treated)

Cell type: CRL 2151 Protein quantity: 5µg

Gel type: 10% Tris-HCL SDSPAGE Electrophoresis Voltage: 100V

Transfer Voltage: 100V Duration: 45min

Primary Antibody: β-actin (abCam) Dilution: 1: 10000 Medium: 5% milk in TBS-T

Secondary Antibody: Anti Mouse Dilution: 1: 10000 Medium: 5% milk in TBS-T

Molecular weight: 42 kDa

Lane 1: ProSieve color protein marker 5µl

Lane 2: RA Day1

Lane 3: DMSO Day1

Lane 4: RA Day2

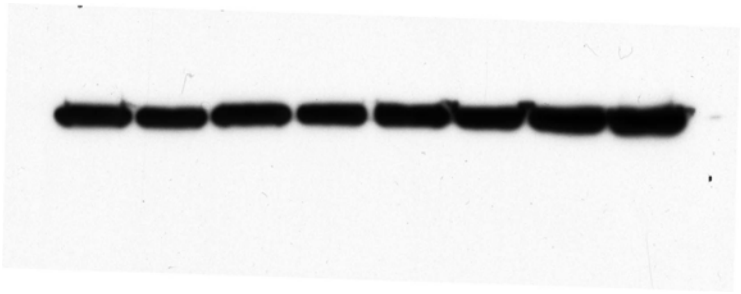
Lane 5: DMSO Day2

Lane 6: RA Day3

Lane 7: DMSO Day3

Lane 8: RA Day4

Lane 9: DMSO Day4



Raw data table produced from spot densitometry of Western blot films.

Chmp1-ATRA treated

Adj. Vol. INT			
Trd-Chmp	Trd-Chmp-actin	Trd-Chmp(ratio)	Trd-Chmp(corr)
2056.11	14398.49	0.142800391	1.0
7115.64	11045.35	0.644220419	4.5
2535.09	13516.78	0.187551325	1.0
799.37	11778.02	0.067869642	0.4
372.59	13409.67	0.027785173	1.0
1563.17	13207.32	0.118356336	4.3
2785.21	16080.38	0.173205484	1.0
3593.7	16593.57	0.216571841	1.3

Film properties report for Stbm (ATRA treated)

Cell type: CRL 2151 Protein quantity: 5µg

Gel type: 10% Tris-HCL SDSPAGE Electrophoresis Voltage: 100V

Transfer Voltage: 100V Duration: 45min

Primary Antibody: StbmN Dilution: 1: 1000 Medium: 5% milk in TBS-T

Secondary Antibody: Anti Rabbit Dilution: 1: 1000 Medium: 5% milk in TBS-T

Molecular weight: 75 kDa

Lane 1: ProSieve color protein marker 5µl

Lane 2: RA Day2

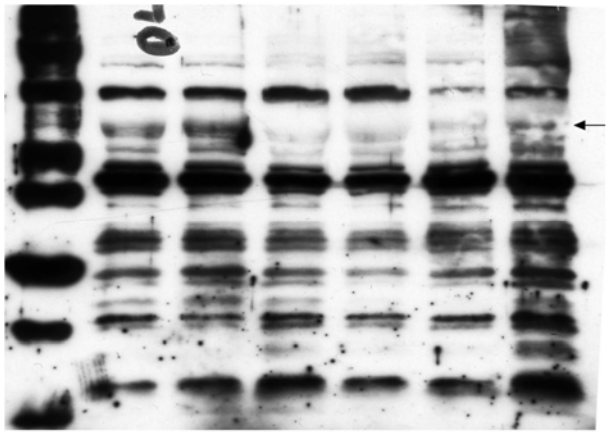
Lane 3: DMSO Day2

Lane 4: RA Day3

Lane 5: DMSO Day3

Lane 6: RA Day4

Lane 7: DMSO Day4



Film properties report for Stbm - βactin (ATRA treated)

Cell type: CRL 2151 Protein quantity: 5µg

Gel type: 10% Tris-HCL SDSPAGE Electrophoresis Voltage: 100V

Transfer Voltage: 100V Duration: 45min

Primary Antibody: β-actin (abCam) Dilution: 1: 10000 Medium: 5% milk in TBS-T

Secondary Antibody: Anti Mouse Dilution: 1: 10000 Medium: 5% milk in TBS-T

Molecular weight: 42 kDa

Lane 1: ProSieve color protein marker 5µl

Lane 2: RA Day2

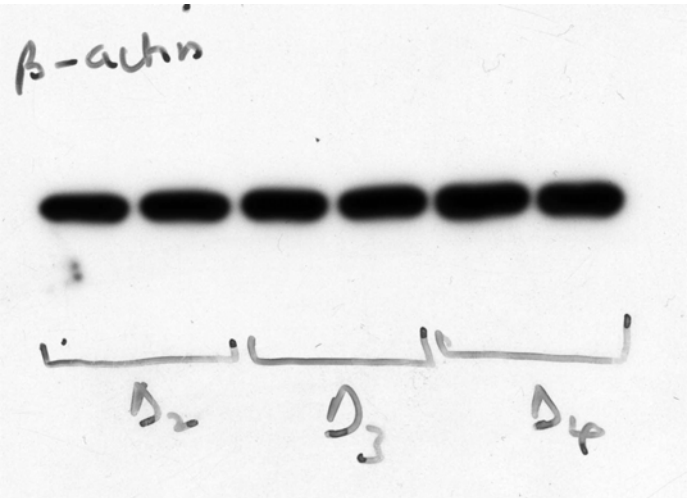
Lane 3: DMSO Day2

Lane 4: RA Day3

Lane 5: DMSO Day3

Lane 6: RA Day4

Lane 7: DMSO Day4



Raw data table produced from spot densitometry of Western blot films.

Strabismus-ATRA treated

Adj. Vol. INT			
Trd-Stbm	Trd-Stbmactin	Trd-Stbm(ratio)	Trd-Stbm(corr)
3533.43	16231.67	0.217687398	1.0
5728.83	17223.41	0.332618802	1.5
645.07	17457.64	0.036950584	1.0
1458.15	18362.55	0.079408906	2.1
811.84	21005.89	0.038648208	1.0
2984.38	16673.09	0.178993816	4.6

Film properties report for p53 (ATRA treated)

Cell type: CRL 2151 Protein quantity: 5µg

Gel type: 10% Tris-HCL SDSPAGE Electrophoresis Voltage: 100V

Transfer Voltage: 100V Duration: 45min

Primary Antibody: p53 (Cell Signaling) Dilution: 1: 1000 Medium: 5% milk in TBS-T

Secondary Antibody: Anti Rabbit Dilution: 1: 1000 Medium: 5% milk in TBS-T

Molecular weight: 53 kDa

Lane 1: ProSieve color protein marker 5µl

Lane 2: RA Day1

Lane 3: DMSO Day1

Lane 4: RA Day2

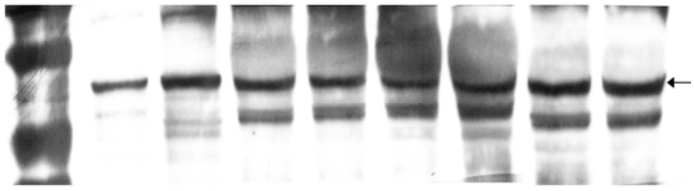
Lane 5: DMSO Day2

Lane 6: RA Day3

Lane 7: DMSO Day3

Lane 8: RA Day4

Lane 9: DMSO Day4



Film properties report for p53- βactin (ATRA treated)

Cell type: CRL 2151 Protein quantity: 5µg

Gel type: 10% Tris-HCL SDSPAGE Electrophoresis Voltage: 100V

Transfer Voltage: 100V Duration: 45min

Primary Antibody: β-actin (abCam) Dilution: 1: 10000 Medium: 5% milk in TBS-T

Secondary Antibody: Anti Rabbit Dilution: 1: 10000 Medium: 5% milk in TBS-T

Molecular weight: 42 kDa

Lane 1: ProSieve color protein marker 5µl

Lane 2: RA Day1

Lane 3: DMSO Day1

Lane 4: RA Day2

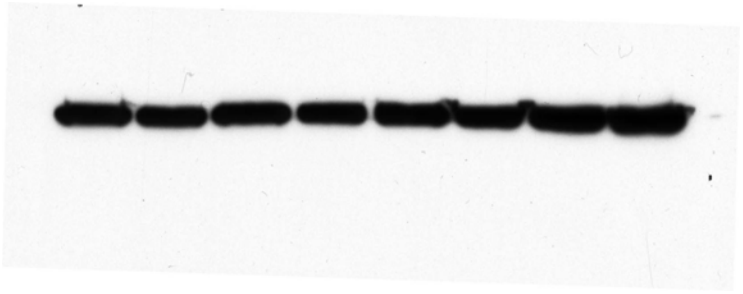
Lane 5: DMSO Day2

Lane 6: RA Day3

Lane 7: DMSO Day3

Lane 8: RA Day4

Lane 9: DMSO Day4



Raw data table produced from spot densitometry of Western blot films.

p53-ATRA treated

Adj. Vol. INT			
Trd-p53	**Trd-p53actin**	**Trd-p53(ratio)**	**Trd-p53(corr)**
1794.39	14398.49	0.12462348	1.0
5120.17	11045.35	0.46355887	3.7
3065.98	13516.78	0.22682769	1.0
2802.92	11778.02	0.23797888	1.0
2292.13	13409.67	0.17093113	1.0
2903.23	13207.32	0.21981977	1.3
3582.19	16080.38	0.22276775	1.0
4262.39	16593.57	0.25686998	1.2

Film properties report for BAD (transfected)

Cell type: CRL 2151 Protein quantity: 5µg

Gel type: 10% Tris-HCL SDSPAGE Electrophoresis Voltage: 100V

Transfer Voltage: 100V Duration: 45min

Primary Antibody: BAD (Cell Signaling) Dilution: 1: 1000 Medium: 5% milk in TBS-T

Secondary Antibody: Anti Rabbit Dilution: 1: 1000 Medium: 5% milk in TBS-T

Molecular weight: 23 kDa

Lane 1: ProSieve color protein marker 5µl

Lane 2: CS2 Day1

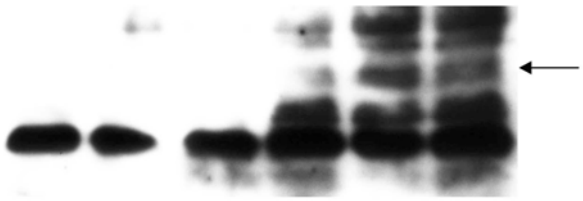
Lane 3: Chmp1 Day1

Lane 4: Stbm Day1

Lane 5: CS2 Day2

Lane 6: Chmp1 Day2

Lane 7: Stbm Day2



Film properties report for BAD- βactin (transfected)

Cell type: CRL 2151 Protein quantity: 5µg

Gel type: 10% Tris-HCL SDSPAGE Electrophoresis Voltage: 100V

Transfer Voltage: 100V Duration: 45min

Primary Antibody: β-actin (abCam) Dilution: 1: 10000 Medium: 5% milk in TBS-T

Secondary Antibody: Anti Mouse Dilution: 1: 10000 Medium: 5% milk in TBS-T

Molecular weight: 42 kDa

Lane 1: ProSieve color protein marker 5µl

Lane 2: CS2 Day1

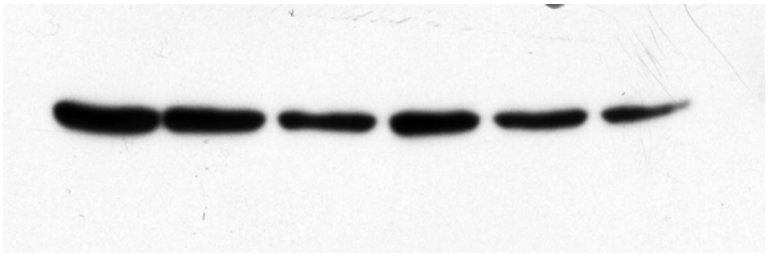
Lane 3: Chmp1 Day1

Lane 4: Stbm Day1

Lane 5: CS2 Day2

Lane 6: Chmp1 Day2

Lane 7: Stbm Day2



Raw data table produced from spot densitometry of Western blot films.

Bad-Transfected

Adj. Vol. INT			
Trf-Bad	**Trf-Badactin**	**Trf-Bad(ratio)**	**Trf-Bad(corr)**
0	12592.14	0	0.0
0.4588	9495.77	4.83163E-05	0.0
0	6162.74	0	0.0
1780.23	8879	0.20049893	1.0
9472.59	6137.55	1.543382946	7.7
8651.35	3726.98	2.321276208	11.6

Film properties report for BAK (transfected)

Cell type: CRL 2151 Protein quantity: 5µg

Gel type: 10% Tris-HCL SDSPAGE Electrophoresis Voltage: 100V

Transfer Voltage: 100V Duration: 45min

Primary Antibody: BAD (Cell Signaling) Dilution: 1: 1000 Medium: 5% milk in TBS-T

Secondary Antibody: Anti Rabbit Dilution: 1: 1000 Medium: 5% milk in TBS-T

Molecular weight: 25 kDa

Lane 1: ProSieve color protein marker 5µl

Lane 2: CS2 Day1

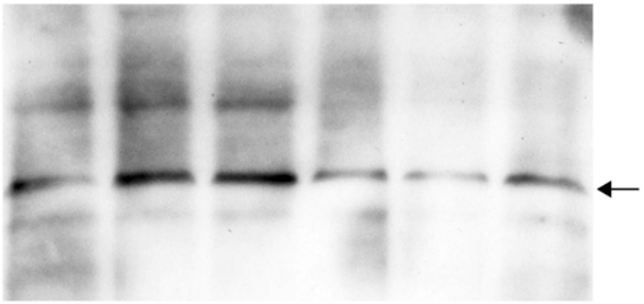
Lane 3: Chmp1 Day1

Lane 4: Stbm Day1

Lane 5: CS2 Day2

Lane 6: Chmp1 Day2

Lane 7: Stbm Day2



Film properties report for BAK- βactin (transfected)

Cell type: CRL 2151 Protein quantity: 5µg

Gel type: 10% Tris-HCL SDSPAGE Electrophoresis Voltage: 100V

Transfer Voltage: 100V Duration: 45min

Primary Antibody: β-actin (abCam) Dilution: 1: 10000 Medium: 5% milk in TBS-T

Secondary Antibody: Anti Mouse Dilution: 1: 10000 Medium: 5% milk in TBS-T

Molecular weight: 42 kDa

Lane 1: ProSieve color protein marker 5µl

Lane 2: CS2 Day1

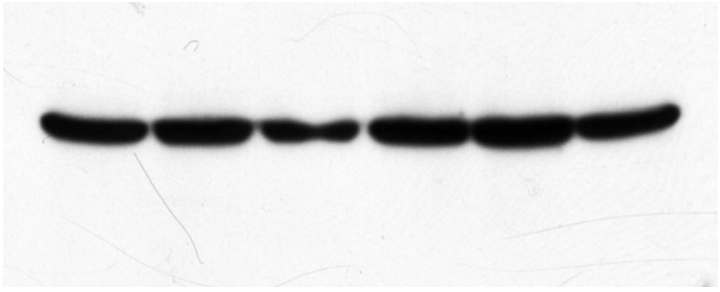
Lane 3: Chmp1 Day1

Lane 4: Stbm Day1

Lane 5: CS2 Day2

Lane 6: Chmp1 Day2

Lane 7: Stbm Day2



Raw data table produced from spot densitometry of Western blot films.

Bak-Transfected

Adj. Vol. INT			
Trf-Bak	**Trf-Bak-actin**	**Trf-Bak(ratio)**	**Trf-Bak(corr)**
2324.67	11910.45	0.19517902	1.0
5273.09	10221.44	0.51588524	2.6
4044.86	9292.91	0.43526301	2.2
1190.51	12478.32	0.09540627	1.0
698.66	14475.16	0.04826613	0.5
1711.21	11878.11	0.14406417	1.5

CURRICULUM VITAE

Juliana Akinsete
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Proctorville, Ohio. 45669
740-886-0625
akinsete1@marshall.edu.

Education

Master of Science, Biology
June, 2006
Marshall University
Department of Biological Sciences
Huntington, WV
Thesis topic: Chmp1 is implicated in the development of pancreatic tumor via the retinoic acid signaling pathway

Master of Science, Food Science and Technology
July, 1998
University of Agriculture
Abeokuta, Ogun state
Nigeria.
Thesis topic: Studies into alternative cooking methods for melon in *ogiri* production.

Bachelor of Science, Food Science and Technology
October, 1989
University of Agriculture
Abeokuta, Ogun state
Nigeria.
Thesis: Evaluation of market place *gari* in Abeokuta, Ogun State

Summary of Research Skills

- Immunoprecipitation
- Silver Staining of Gel
- Agarose Gel Electrophoresis
- Mini and Midi Prep of DNA
- RNA Isolation from Cells
- Western Blot
- Cell culture
- PCR

Computer Skills

- Microsoft Windows (2000)
- Microsoft Office (2003) – Microsoft Word, Microsoft Woks, Power Point, and Microsoft Excel

Work Experience

Teaching Assistant
January – April 2005
Department of Biological Sciences
Marshall University
Huntington, WV

Duties:

- Teach Introductory Biology (BSC 104) laboratory classes
- Tutorials in Introductory Biology (BSC 104)

Research Associate
May – April 2006
Dr. Maiyon Park.
Department of Biochemistry
School of Medicine
Marshall University
Huntington, WV

Duties:

- Perform experiments

College Professor
September 1993 – August 2001
Moshood Abiola Polytechnic
Abeokuta, Ogun State
Nigeria

Duties:

- Teach courses in food processing and preservation, nutrition, nutritional biochemistry, food microbiology, food packaging, food quality control and food plant sanitation

- Supervise students' projects

Agricultural Extension Officer
November 1991 – September 1993
Ogun State Agricultural Development Project
Ogun State
Nigeria

Duties:

- Conduct workshops and trainings for rural women in food processing and preservation

Facilitator (National Youth Service Corps)
September 1989 – August 1990
Ogun State Agricultural Development Project
Ogun State
Nigeria

Duties:

- Conduct workshops and trainings for rural women in food processing and preservation

Co-op Experience
January 1988 – August 1988
Standard Breweries Nigeria Limited
Ibadan, Nigeria

Duties:

- Conduct experiments in the Quality Control laboratories
- Work on the processing line

Co-op Experience
April 1987 – July 1987
Ogun State Agricultural Development Corporation
Ogun State
Nigeria

Duties:

- Conduct experiments in the Quality Control laboratories
- Work on the processing line

Award

- 1996/1997 Federal Government of Nigeria Postgraduate Award - University of Agriculture, Abeokuta, Nigeria

Presentation at Conferences and professional meetings

- Akinsete, J.A. {2000}. "Effect of pressure-cooking on the chemical component of *ogiri*". Presented at the 24th Annual Conference of Nigerian Institute of Food Science and Technology (NIFST). Bauchi, Bauchi State, Nigeria.

- Akinsete, J.A. and Oyewole, O.B. (2000). "Microbiological safety of *ogiri* produced from fermented melon (*citrullus vulguris)*". Presented at the 24th Annual Conference of Nigerian Institute of Food Science and Technology (NIFST). Bauchi, Bauchi State, Nigeria.

- Agbotoba, M.O. and Akinsete, J.A (2000). "Effect of various debittering methods on the sensory attributes of the bitter leaf (veronia amygdalina) soup". Presented at the 24th Annual Conference of Nigerian Institute of Food Science and Technology (NIFST). Bauchi, Bauchi State, Nigeria.

- Akinsete, J.A. (1997) "Effects of microwave cooking on the nutritional quality and acceptability of fermented melon". Presented at the 21st Annual Conference of Nigerian Institute of Food Science and Technology (NIFST). Uyo, Akwa Ibom State, Nigeria.

- Akinsete, J.A. (1996) "Assessment of spiced *robo*- A local melon snack". Presented at the 20th Annual Conference of Nigerian Institute of Food Science and Technology (NIFST). Ikeja, Lagos State, Nigeria.

Workshop attended

- "Soy-bean Popularization Project". Federal Ministry of Agriculture, Water Resources and Rural Development, University of Agriculture, Abeokuta, Nigeria. April, 1993.

- "Strategies for Mobilizing Rural Women for Economic Development". Agricultural Media Resources and Extension Center (AMREC), University of Agriculture, Abeokuta, Nigeria. May, 1992.

- "Storage Technologies and Pest Control of Produce". Presidential project on on-farm storage of produce. Organized by the Nigerian Stored Product Research Institute, held at the Ogun State Agricultural development Project, Abeokuta, Nigeria. November, 1992.

Services

Professional:

- Treasurer, Organizing Committee for the 23rd Annual Conference/General Meeting of Nigerian Institute of Food Science and Technology. 1999.

- Member, Organizing Committee for the 10th Annual Symposium of Nigerian Institute of Food Science and Technology. 1996.

Academic:

- **Chairperson –** Accreditation Committee. Coordinating activities and preparations toward the accreditation of the diploma program in the department program in the Department of Food Science and Technology, Moshood Abiola Polytechnic, Abeokuta, Nigeria. 1999-2000.

- **Officer-in-Charge –** Preparation of Lecture Time Table and Examination for the Department of Food Science and Technology, Moshood Abiola Polytechnic, Abeokuta, Nigeria. 1996-1998.

- **Officer-in-Charge –** Registration of students in the Department of Food Science and Technology, Moshood Abiola Polytechnic, Abeokuta, Nigeria. 1994-1996.

- **Acting Chair –** Department of Food Science and Technology. Coordinating the activities of the chair in the absence of the chair.

Community:

- **Volunteer –** Place of Hope Ministries. Assist in catering for the homeless. St. Cloud, Minnesota, 2003.

- **Member –** Editorial Board for Academic Staff Union of Moshood Abiola Polytechnic, Abeokuta Chapter Newsletter. 1999

- **Member –** Ceremonial Committee. Organizing the convocation and related events in Moshood Abiola Polytechnic, Abeokuta, Nigeria. 1998.

- **Member –** Development Fund Committee: Organizing the development fund raising activities for Moshood Abiola Polytechnic, Abeokuta, Nigeria. 1998.

- **Member** – Investigating Panel. Investigating students' misconduct during examination at Moshood Abiola Polytechnic, Abeokuta, Nigeria. 1997.

Professional Affiliation

- Nigerian Institute of Food Science and Technology, (1994).

Extra-curricular activities

- Reading, Singing, and Traveling.