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DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

A STUDY OF OKL38, A POTENTIAL TUMOR SUPPRESSOR, IN MITOCHONDRIA STRUCTURE AND FUNCTION

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Abstract

The mitochondria of eukaryotic cells play an important role in metabolic functions as well as controlling the programmed cell death termed apoptosis. Many tumor suppressing proteins have been identified to date. One of the best studied is p53. This protein has many proapoptotic target genes, including OKL38. OKL38 translocates to the mitochondria following DNA damage or oxidative stress, inducing apoptosis. The mechanism through which this protein achieves its function is currently unknown. Inducible OKL38 expression has been achieved in MCF7 cells and Western Blot analysis shows that a small amount of endogenous OKL38 exists in these breast cancer cells. Phage display and plaque assays have revealed a group of potential OKL38 target proteins, including p53 and subunits of the electron transport chain.

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Chapter 1. Introduction

Over time, eukaryotic organisms evolved a complex organelle to assist in metabolic processes, the mitochondria. Bound by an inner and outer membrane, the main function of mitochondria in animal cells is to produce the majority its ATP. The inner most space, referred to as the matrix, contains enzymes that facilitate the breakdown of pyruvate and fatty acids into acetyl CoA and the oxidation of acetyl CoA to CO_2 in the citric acid cycle. These reactions produce the electron carriers NADH and $FADH_2$, which are used in the electron transport chain located in the inner membrane[1].

The electrons provided by NADH and $FADH_2$ are used to pump protons out of the matrix, creating an electrochemical proton gradient across the inner membrane. When H^+ flows back into the matrix, a large amount of free energy is release, facilitating the production of ATP through ATP synthase. Along with protons, the transmembrane electrochemical gradient aids the active transport of ATP, maintaining a high concentration of ATP in the cytosol. This high ratio of ATP to ADP makes ATP hydrolysis energetically favorable, allowing for this reaction to power many energy requiring cell processes[1].

Another important function of the mitochondria is the regulation of apoptosis. Apoptosis is the most common form of programmed cellular death. It is characterized by morphological changes such as cell shrinking, collapse of the cytoskeleton, disassociation of the nuclear envelope, and the condensation and fragmentation of nuclear chromatin[1]. The most widely accepted model of mitochondrial mediated apoptosis describes the mitochondria undergoing

extensive fragmentation before the caspase family of proteases is activated, which break down many proteins in the cell[2].

The release of cytochrome c into the cytosol is one of the main characteristics of apoptosis and occurs after the fragmentation of the mitochondria. BAX and BAK, members of the BCL2 family of proteins, form pores in the outer mitochondrial membrane, allowing cytochrome c to be released into the cytosol. After it has left the mitochondria, cytochrome c activates and binds to Apaf-1, forming a heptamer termed an apoptosome which activates procaspase-9[3]. Once this protein initiates apoptosis, neighboring cells or macrophages rapidly engulf them to prevent any of their contents from leaking out[1].

BAK and BAX are both crucial to changing the outer membrane permeability. While it was previously thought that these two proteins served the same function, some studies have shown that BAK and BAX have unique roles in apoptosis[4]. BAK has shown the ability to mediate mitochondrial fragmentation independent of BAX. However, BAK alone is not sufficient to induce cytochrome c release. BAK and BAX must work together to induce complete release of cytochrome c[4]

Along with the BCL2 family, many other proapoptotic proteins have been identified. One of the most well known and widely studied protein is p53. It has been observed in the majority of human cancers that p53 is functioning improperly. When working correctly, p53 acts as a tumor suppressing protein. It can be activated in response to stress such as DNA damage, growth signals triggered by the activation of oncogenes such as Ras, protein kinase-inhibitors,

chemotherapeutic drugs, and ultraviolet radiation. All of these factors inhibit the degradation of p53, keeping it at sufficient concentrations to allow it to bind to and activate its downstream target genes[5]. Activation of the p53 protein is dependent on posttranslational modifications such as phosphorylation, ubiquitination, methylation, and acetylation of p53. Different types of DNA damage will signal different modifications to the protein[6].

Increased expression of p53 leads to cell cycle arrest in mammalian cells. The p53 protein works directly to activate the expression of another tumor suppressing gene, p21. The protein product of this gene inhibits cyclin dependant kinases. Inhibition of CDKs does not allow a cell to proceed from the G_1 phase to the S phase of the cell cycle, where most of the DNA synthesis necessary for division takes place. p21 can also arrest cells in the G_2 phase before it enters M phase and divides. Both of these actions ensure that damaged cells do not proliferate [5].

Along with cell cycle arrest, p53 can also influence cells to undergo apoptosis. Many target genes have been identified that could be activated by p53 when the protein binds to specific DNA sequences[6]. In human cells, p53 has been shown to directly activate BAX[5]. Other genes have also been implicated in being activated by p53, such as NOXA and PUMA, which facilitate the release of cytochrome c from mitochondria. Another possibility is that p53 promotes apoptosis in a transcription-independent manner by direct interaction with the mitochondria causing it to produce an excess of reactive oxygen species[5,6]. In response to DNA damage, translocation of p53 from the nucleus to the mitochondria has shown apoptotic responses before the activation of any of its target genes[6].

One target of p53 that has been described as having tumor suppressing properties is OKL38. While present in most tissues, OKL38 is expressed in relatively high levels in the ovaries, kidneys, and liver and down regulated in most cancers[7,8]. It is classified as a pregnancy induced growth inhibitor because it is found to have increased expression during pregnancy and lactation[7]. After DNA damage, levels of p53 increase at the OKL38 promoter. Upon over expression, OKL38 translocates to the mitochondria, inducing morphological changes and subsequently, apoptosis[8].

In undamaged cells, the expression of OKL38 is inhibited by a protein known as peptidylarginine deiminase 4 (PAD4). This enzyme has been described in humans as not only converting arginine residues to citrulline residues, but also can remove methyl-arginine in a demethylimination reaction[9]. It is in this manner that PAD4 is able to regulate the expression of OKL38. Under normal conditions, PAD4 association as well as histone citrullination at the OKL38 promoter are high. Following DNA damage both of these factors are considerably lower and OKL38 expression is increased, suggesting that PAD4 plays an repressive role in regulating the activity of OKL38[8]. PAD4 has also been shown to inhibit the expression of other p53 target genes such as PUMA and p21[10].

My thesis study takes a closer look at the specific function of OKL38 as a tumor suppressor in the mitochondria. While it is known that OKL38 translocates to the mitochondria following DNA damage and induces apoptosis[8], it remains unclear how it interacts with the mitochondria in order to do so. Inducing OKL38 expression in MCF7 cells will give insight into whether or not over expression of OKL38 alone is enough to induce apoptosis. Examining

potential interacting proteins of OKL38 in mitochondria will provide useful insight into the pathway this protein takes to inhibit tumor growth.

Chapter 2. Methods

Establishing a stable MCF-7 cell line with OKL38 expression:

To establish a stable cell line with OKL38 expression, MCF-7 cells were transfected with an OKL38 plasmid using the TET-On system. MCF-7 cells were cultured on a plate with fresh medium. OPTI-MEM (250µl) medium and lipofectamine 2000 (10µl) were combined in one microcentrifuge tube. In another tube, OPTI-MEM (250µl) and the OKL38 plasmid (4µg) were added and mixed. The contents of both tubes were then combined and let sit for 20 minutes at room temperature. After this time, the medium of the MCF-7 cells was changed and the plasmid/lipofectamine mixture was added to the culture. For the next 96 hours, the medium was changed once every 24 hours. To select for the transfected cells, the culture was treated with neomycin (50mg/ml) and zeocin (5mg/ml), incubated at 37^{o}C for 24 hours, and treated again. Finally, the cells were treated with doxycycline (1µg/ml) to induce OKL38 expression and incubated over night. Western Blot analysis was used to test the cells for protein expression.

Phage Display Analysis:

A phage display analysis was conducted in order to identify potential target proteins of OKL38 in the mitochondria. The original library was a T7 human liver cDNA phage library provided by Novagen. In order to amplify the library, 5615-E. coli was cultured in LB broth (3ml) with ampicillin and shaken at 37^{o}C overnight. His6-OKL38 was diluted (10µg/ml), and applied to a freshly prepared ELISA plate (100µl). This plate was covered and incubated at 4^{o}C overnight. The culture (1ml) was then added to LB/ampicillin medium (50ml) and allowed to grow to an OD_{600} of 0.569. IPTG (1mM) was added when the OD_{600} reached 1.177 and shaken for 30 minutes. The culture was infected with the phage library. The culture was then shaken at

37^{o}C until lysis was observed (100 minutes). Lysis is characterized by the accumulation of long thin strands of debris in the medium. The mixture was spun (8000g for 10 minutes) and the supernatant decanted into a sterile 1.5ml tube. PEG 8000/NaCl was added and the mixture was incubated at 4^{o}C for 1 hour. After this time the mixture was centrifuged (8000g for 20 minutes), and the pellet was kept and dissolved in TBS (500µl).

To titer the phage library, dilutions of the original phage sample ($1.0x10^{10}$ pfu/ml) were prepared for the first input of the plaque assay. The phage was diluted to a dilution factor of 10^{8}-10^{12} for the first input. Multiple dilutions were prepared because it was unknown before they were made which dilution would produce an appropriate number of colonies (between 10 and 200) to be used for the subsequent round of biopanning. A mixture of the appropriate phage dilution (100µl), host cells (250µl), IPTG (1M, 10µl), and top agarose (3ml) was prepared for plating. Plates (LB/amp^{+}) for each dilution were prewarmed at 37^{o}C for 30 minutes before the application of the mixture. Once the agarose had hardened, the plates were incubated for 1 hour at 37^{o}C. The phages were then counted for each plate and the phage titer was calculated (described in plaque forming units or pfu). Phages of interest were then scraped off of the agarose gel, dispersed in phage extraction solution (100µl) and incubated at 4^{o}C overnight.

An ELISA plate was prepared by treating it with a 5% blocking reagent (5% BSA in TBS) and incubating for 1 hour at room temperature. The amount of phage lysate to be added to the plate was calculated by dividing the number of clones of the cDNA library to be screened by the titer of the amplified library. If the volume of lysate to be added was less than 100µl, 1X TBST was added to make the total volume added equal to 100µl. The plate was then incubated at 4^{o}C overnight.

After the incubation period, the plates were washed with 1X TBST, the bound phage was

eluted using the T7 Elution Buffer (200µl), and incubated at room temperature for 20 minutes. The eluted phage was then transferred to a sterile 1.5ml tube and 10µl were used as the first output. The remaining eluted phage (100µ) was plated and prepared in the same fashion as the first input and was used as the second input with dilution factors of 10^5-10^8. The second output was prepared in the same manner as the first output with dilution factors of 10^3-10^6. This process was repeated until a fifth output was collected.

PCR was run to amplify the amount of DNA obtained from the fifth output. The phage lysate (2µl) was combined with 10X *Taq* Buffer with $MgCl_2$ (5µl), T7 Select UP Primer (1µl), T7 Select DOWN Primer (1µl), dNTP mixture (1µl), *Taq* DNA polymerase (1.25U), and deionized water (40µl). The reaction was heated to 80^{o}C before the addition of DNA polymerase. A total of 35 cycles were run as follows: 94^{o}C for 50 seconds, 50^{o}C for 1 minute, 72^{o}C for 1 minute, and a final extension of 72^{o}C for 6 minutes. Once amplified, gel electrophoresis was run to determine the size of the 100 samples (Figure 3). Samples of the same size were grouped together and ones of relevant size were sent for sequencing. Samples that were the same size were hypothesized to be the same protein. To qualify as a potential target, the DNA fragments needed to be between 200 and 600bp because this parameter fit the size of the phage insert, making it more likely to be a positive result. Once the sequence was obtained, Blastn and Blastx databases were used to ascertain the gene as well as the protein product these sequences coded for.

Chapter 3. Results

Expression of OKL38 in MCF-7 Cells

To establish a stable MCF-7 cell line with OKL38 expression, the breast cancer cells were transfected with an OKL38 plasmid with the TET-On system. This system makes use to doxycycline to initiate transcription of a gene of interest. Upstream of the promoter there is a DNA binding domain where doxycycline will bind and promote transcription. Downstream of that are three minimal transcription activation domains. Next lies the gene of interest which is transcribed. In this case, the genes to be transcribed are FLAG-HA-OKL38. Upon treatment with doxycycline, the expression of this gene is induced (Figure 1).

Western blot showed differential OKL38 expression between MCF-7 cells treated with doxycycline and those transfected with the FLAG-HA-OKL38 plasmid but not treated (Figure 2). Cells that received doxycycline treatment expressed more OKL38 than those that did not receive treatment. Both treated and untreated cells showed expression of OKL38. Interestingly, the α-OKL38 antibody detected two bands on western blot, one at approximately 55kDa and another at approximately 43kDa . Both bands showed the same pattern of expression when given treatment versus not being treated. The α-Flag-HA antibody also showed two bands, one at 100kDa and another at 73kDa. The 100kDa band showed the same level of expression with or without doxycycline treatment whereas the 73kDa band showed reduced expression when treated with doxycycline. The two bands detected by the FLAG-HA antibodies did not correspond to the OKL38 bands, suggesting there was non-specific recognition. Future work needs to be performed to analyze OKL38 expression in the FLAG-HA-OKL38 inducible MCF7 cells.

Identification of Potential OKL38 Target Proteins

To identify potential OKL38 target proteins, a phage display analysis was performed using human liver cDNA and His6-OKL38. After each round of biopanning, plaques from one dilution were selected to be used for the next round of biopanning and subsequent dilutions. In the first input, the sample diluted to 10^8 produced 45 plaques ($4x10^{10}$ pfu/ml) and was used for the first output. This process was repeated until a fifth output was produced ($1.6x10^7$ pfu/ml). After five rounds of biopanning, 100 colonies were taken from the fifth output and run through gel electrophoresis (Figure 4). Thirty six of these colonies were identified with appropriate length of inserts as potential OKL38 interacting partners and sequenced. Of the 36 colonies, 30 produced at least one gene or protein match to the acquired nucleotide sequence when run through BLASTn and BLASTx (Table 1).

Particularly interesting were candidate proteins that are involved in mitochondrial functions. Cytochrome b, cytochrome c subunits I and II, NADH dehydrogenase subunit II, and ATP synthase were identified from the selected colonies. These enzymes are all incorporated into the inner mitochondrial membrane and involved in oxidative phosphorylation[1] (Figure 5). Cytochrome b and c make up Complex III, cytochrome c oxidase, of the electron transport chain. Its purpose is to transfer electrons from cytochrome c to oxygen. What makes this enzyme of particular interest to this study is that cytochrome c release from the mitochondria is observed when a cell undergoes apoptosis.

NADH dehydrogenase is another enzyme involved in the electron transport chain. The first step of the electron transport chain, it accepts electrons from NADH and transfers them to ubiquinone[1]. Some forms of cancer have been linked to mutations in this enzyme that lead to

the generation elevated levels of reactive oxygen species which is highly toxic to cells[11]. ATP synthase is the last step of oxidative phosphorylation and makes use of the proton gradient created by the preceding steps to make ATP. OKL38 binding to any of these enzymes could lead to the initiation of apoptosis by impacting on the oxidative phosphorylation process.

Albumin was also a protein of interest because of its high prevalence in the liver and the cDNA library used in this experiment being human liver DNA. It is made in the liver and assists in the transport of highly insoluble molecules across the blood stream. Its presence as a potential OKL38 target shows that there could be unspecific binding in the phage display. Other proteins identified such as Apolipoprotien E and Collagen type XVIII (Table 1) could also be due to unspecific binding. However, the nature of the phage display does not allow for any of these potential targets to be excluded as OKL38 targets.

Chapter 4. Discussion

Western Blot analysis showed that OKL38 expression was able to be induced in MCF7 cells. However, the results also suggest that a low amount of endogenous OKL38 exists as illustrated by OKL38 expression being present without doxycycline treatment. The doxycycline was used to turn on the promoter and stimulate expression of OKL38. The presence of OKL38 without doxycycline indicates that it is being expressed in MCF7 cells and that the transfection served to over express this protein. Further study into how the over expression of OKL38 in MCF7 cells effects cell growth and apoptosis could reveal whether OKL38 expression alone is enough to inhibit tumor growth.

The presence of two bands close to but not at the exact molecular weight of OKL38 (54kDa) was surprising. One explanation could be due to the position of methionine coding regions along the gene. Methionine codons flank the Flag-HA on both sides and lie within OKL38. It is possible that another methionine codon exists within the OKL38 portion of the gene, initiating translation downstream of the actual starting point of the entire protein, accounting for the separate bands. While the first methionine codon is usually used as the starting point of translation in eukaryotic cells, some instances have been observed where it is ignored and a methionine downstream is used to initiate translation. After mRNA is transcribed, the 40S ribosomal subunit binds at the 5’ end and begins translation at the first AUG it encounters. The positioning of a purine three nucleotides upstream and a guanine four nucleotides downstream of the start codon increases the efficiency of initiating translation. The

absence of these conditions can cause a ribosome not to recognize this codon as the starting point and move on to the next AUG[12].

Many of the proteins identified through the phage display analysis could provide some insight into the behavior of OKL38 as it induces apoptosis. One interesting target that was identified is p53. The relationship between p53 and OKL38 at the promoter level has been previously well documented[7,8]. DNA damage induces p53 to bind to the OKL38 promoter and increase protein expression. While it is known that OKL38 translocates to the mitochondria[8], it is unclear how it accomplishes this. Some studies have also suggested that p53 may directly stimulate the mitochondria to induce apoptosis[5]. The results of this experiment show that p53 may also interact with the OKL38 protein and not just the promoter. Taken together, this could suggest that p53 and OKL38 translocate to the mitochondria together or interact with each other once they have reached the mitochondria. Immunostaining of cells positive for both OKL38 and p53 expression could discern if the proteins colocalize at the mitochondria during apoptotic events.

Another interesting result gathered from the phage display analysis was the identification of four subunits of the electron transport chain: cytochrome b, cytochrome c oxidase subunits I and II, NADH dehydrogenase subunit II, and ATP synthase. The electron transport chain is involved in the transfer of electrons from molecules such as NADH and $FADH_2$ produced from the TCA cycle and β-oxidation of fatty acids to oxygen during oxidative phosphorylation. The main purpose of these enzymes is to make ATP and provide a constant energy source for the cell. There are five subunits in total, each contains numerous polypeptides chains of varying lengths.

All of the enzymes of the electron transport chain are contained within the inner mitochondrial membrane[1].

A hallmark of apoptosis is the release of cytochrome c from the mitochondria. Cytochrome c oxidase is also one of the electron transport chain subunits. Its function is to accept an electron from cytochrome c and transfer electrons four at a time to oxygen. This enzyme consumes approximately 90% of the oxygen uptake in eukaryotic cells[1]. From the results of the phage display, it is plausible that OKL38 interacts with cytochrome c oxidase in such a manner that facilitates the release of cytochrome c during apoptosis after the membrane permeability has changed. Studying the possible synergy between BAK, BAX, and OKL38 would be useful in determining if the proposed interaction is meaningful.

NADH dehydrogenase is the largest of the subunits and is involved in the first step of the electron transport chain. As its name implies, this enzyme accepts electrons from NADH and transfers them to ubiquinone[1]. Mutations in this enzyme have been reported in cases of cancer. Most of these mutations lead to the generation elevated levels of reactive oxygen species which is highly toxic to cells[11]. It is possible that OKL38 can interact with NADH dehydrogenase in such a manner that also leads to the accumulation of deadly ROS as a result of DNA damage. Flow cytometry analysis after over expression of OKL38 could be used to detect if OKL38 leads to a buildup of ROS in cells induce apoptosis.

Another point of interest stemming from this study is the idea of apoptosis induction due to oxidative stress. The electron transport chain is not a perfectly efficient system, occasionally

leaking oxygen radicals. An excess of these radicals can lead to a deadly build up of ROS, inducing apoptosis. Once oxygen has picked up an electron, cytochrome c oxidase binds to it through its heme center until it has acquired three more electrons[1]. A disruption of any portion of the electron transport chain could prove fatal for a cell due to a buildup of toxic ROS. The proposed interaction of OKL38 with the electron transport chain could cause enough oxidative stress to the cell to induce apoptosis.

The number of potential matches and the frequency of albumin being sequenced leads to the conclusion that there could have been a lot of non-specific binding between the phage and the cDNA library that is not indicative of OKL38 interactions. Albumin is a protein that aids in increasing the solubility of molecules such as fatty acids and carriers them across the blood stream. Human serum albumin is secreted in large quantities from the liver[1]. The T7 select phage library used for this experiment contained cDNA from human liver cells. The origin of the cells could account for the higher frequency of albumin matches than any of the other proteins identified. The presence of apolipoprotein E, a target of albumin, could also be explained in this manner.

If unspecific binding is present, the exclusion of a final biopanning step could be an explanation. The T7 select protocol provided by Novagen used for this experiment offered a biopanning kit that included reagents with large target ligands using 96-well microtiter plates. In an effort to save on costs, this kit was not purchased under the assumption that 5 rounds of biopanning would yield only proteins that interact with OKL38. While all of these factors may suggest unspecific binding was present, nothing in this experiment can eliminate the possibility

that all of the matches do in fact directly interact with OKL38. Further testing would need to be conducted to test whether the proteins identified in this experiment interact with OKL38 or were identified due to the nature of the liver cDNA library used.

Another assumption of this experiment was that clones of the same molecular weight would be the same protein. This hypothesis turned out not to be incorrect, as most of the protein matches were not repetitive. To further complicate matters, very primitive imaging technology and the naked eye were used to determine the molecular weight of each sample, and the accuracy of estimation was only 100bp. Samples that were listed as the same size most likely had different molecular weights that would be undetectable using the methods of this study.

This study of the behavior of OKL38 provides valuable insight into how this protein possibly promotes apoptosis. Based on these results it is possible to induce OKL38 expression in MCF7 cells, but a small amount of endogenous protein does exists. While it is known that OKL38 translocates to the mitochondria following DNA damage and preceding apoptosis, it is unclear how it accomplishes this function. This study presents a list of possible target proteins, some of which are not only mitochondrial proteins but could also induce apoptosis via oxidative stress and cytochrome c release. It was also revealed that p53 may interact with the OKL38 protein, not just the promoter region, and play an active role in OKL38 mediated apoptosis. Further investigation is warranted to rule out proteins that could have been false positives and whether or not OKL38 interaction with the subunits of the electron transport chain causes an increase in the accumulation of toxic reactive oxygen species, leading to apoptosis.
1.0x1010pfu/ml

Figures

Sample Number and Size (bp)	Blastn	Blastx
2 (500)	Eukaryotic translation elongation factor 1 alpha 1	Eukaryotic translation elongation factor 1 alpha 1
8 (400)	Glutamic-oxaloacetic transaminase 1	Glutamic-oxaloacetic transaminase 1
9 (500)	No Matches	Zinc finger protein 30 homolog
16 (500)	Tripartite motif-containing 38	No Matches
17 (500)	18S ribosomal RNA	No Matches
18 (600)	Insulin-like growth factor binding protein 3	No Matches
24 (500)	mitochondrion, complete gene	1. ATP synthase F0 subunit 6 2. ATPase subunit 6
28 (600)	Starch binding domain 1	Starch binding domain 1
31 (600)	Albumin	Albumin
36 (500)	No Matches	No Matches
42 (600)	Tumor protein p53 inducible nuclear protein 1	No Matches
51 (500)	Similar to NADH dehydrogenase subunit II	NADH dehydrogenase subunit II
57 (500)	Ornithine aminotransferase, nuclear gene encoding mitochondrial protein	Ornithine aminotransferase
58 (200-300)	Albumin	Albumin
60 (400)	4-hydroxyphenylpyruvate dioxygenase	4-hydroxyphenylpyruvate dioxygenase
63 (200-300)	Similar to hCG2003116	No Matches
64 (600)	Albumin	Albumin
65 (400-500)	Deiodinase, iodothyronine, type I	No Matches
71 (400)	Similar to cytochrome c oxidase subunit II	Cytochrome c oxidase subunit I
72 (400)	Adaptor protein, phosphotyrosine interaction, pH domain and leucine zipper containing 1	Adaptor protein, phosphotyrosine interaction, pH domain and leucine zipper containing 1
74 (500bp)	No Matches	No Matches
75 (200-300)	Mitochondrion, complete genome	Cytochrome b
77 (400-500)	Heat shock protein 90KDa alpha, class B member 1	Heat shock protein 90KDa alpha, class B member 1
79 (500)	Albumin	Serum Albumin
80 (500)	Selenoprotein P, plasma, 1	Selenoprotein P, plasma, 1
85 (400-500)	Similar to cytochrome c oxidase subunit II	No Matches
91 (400-500)	DEAD box polypeptide, X-Linked	No Matches
92 (400-500)	Apolipoprotein E	Apolipoprotein E
97 (400-500)	Collagen type XVIII, alpha 1	Collagen type XVIII, alpha 1
98 (500)	Aldolase B, fructose-biphosphate	Aldolase B, fructose-biphosphate

Table 1: List of candidate proteins

The above table lists potential proteins OKL38 interacts with through the phage display. Blastn results indicate the gene a given sequence matched and Blastx results indicate the protein product resulting from that sequence. Only samples that had at least one match are listed. A total of 36 samples were sequenced. Samples were chosen based on the length of the phage insert (200-600bp).

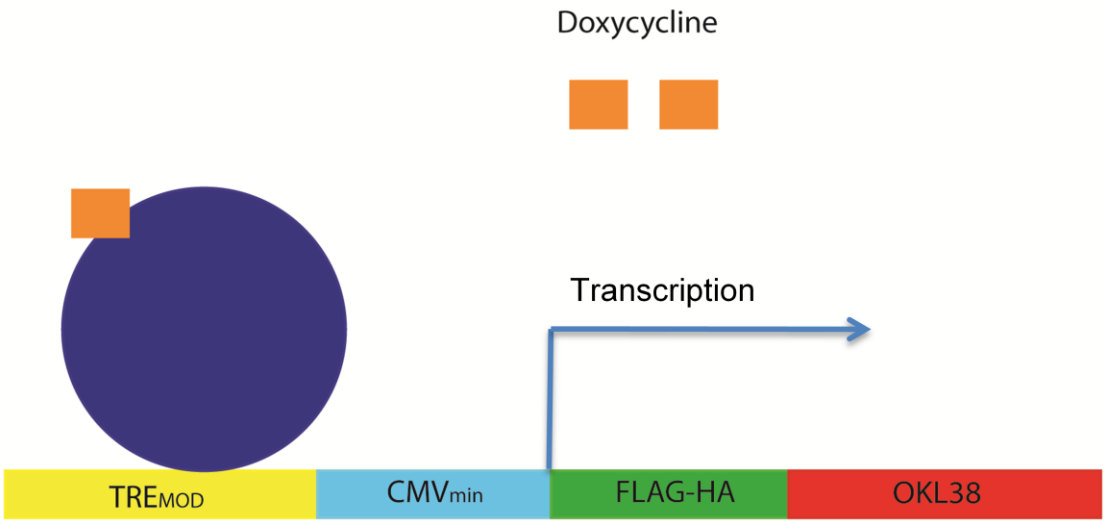

Figure 1: Model of Doxycycline Induced Gene Expression
This construct is composed of the recognition sequence for TET-On (TRE_{MOD}), three core promoter domains (CMV_{min}), and the gene to be transcribed (FLAG-HA and OKL38). Doxycycline binds to the DNA binding domain and initiates transcription.

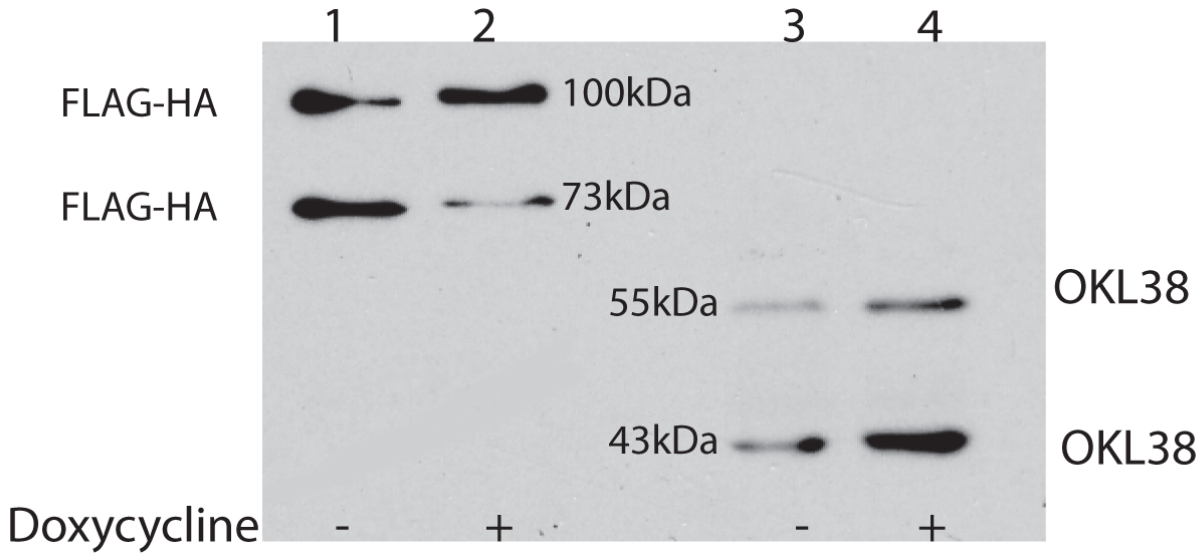

Figure 2: Western Blot of Inducible OKL38 Expression MCF7 TET-On Cells
The two bands in lanes 1 and 2 molecular weights 100kDa and 73kDa from top to bottom and were tagged with α-Flag-HA antibody. The two bands in lanes 3 and 4 are molecular weights 55kDa and 43kDa from top to bottom and were tagged with α-OKL38 protein. Samples in lanes 1 and 3 were taken from cells that did not receive doxycycline treatment while samples from lanes 2 and 4 were taken from cells that did receive the treatment.

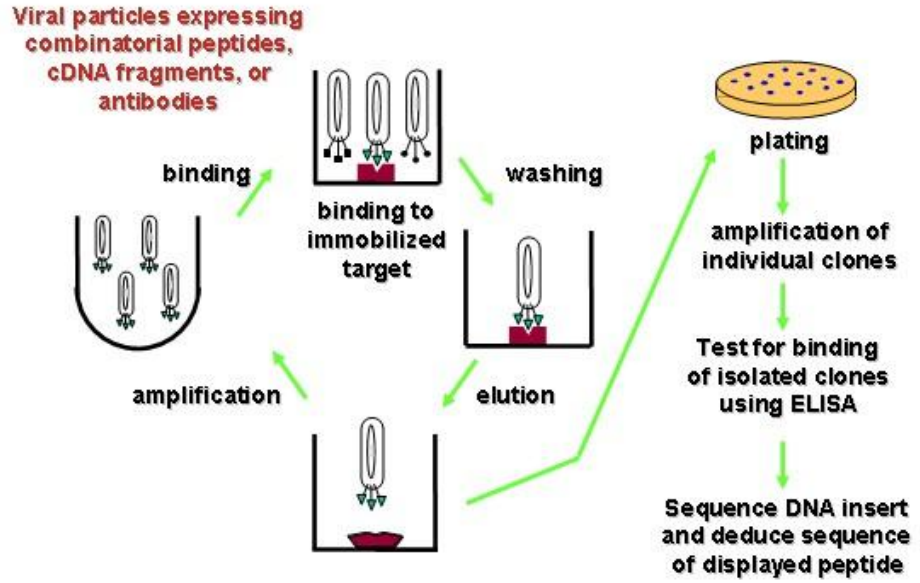

Figure 3: Schematic View of a Phage Display[13]
A phage display was used to identify proteins that potentially interact with OKL38. In a phage display, the protein of interest (OKL38) is ligated and the phage is inserted into E. Coli host cells. The protein will then interact with immobilized targets and non-binding proteins are washed away.

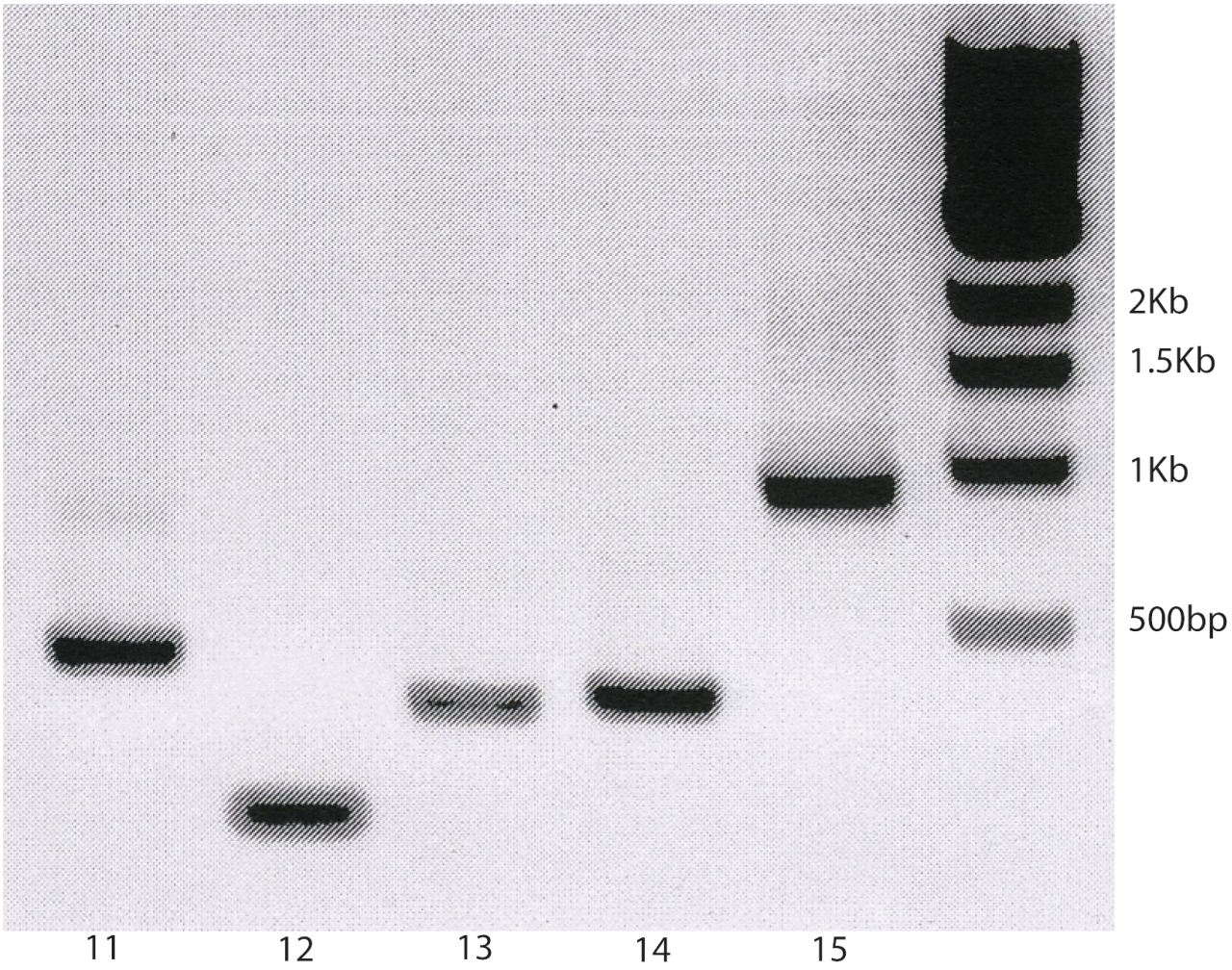

Figure 4: DNA gel from fifth output of Phage Display
Gel electrophoresis was run on 100 samples from the fifth output of the phage display. This figure depicts the DNA gel run on the PCR products for samples 11-15.

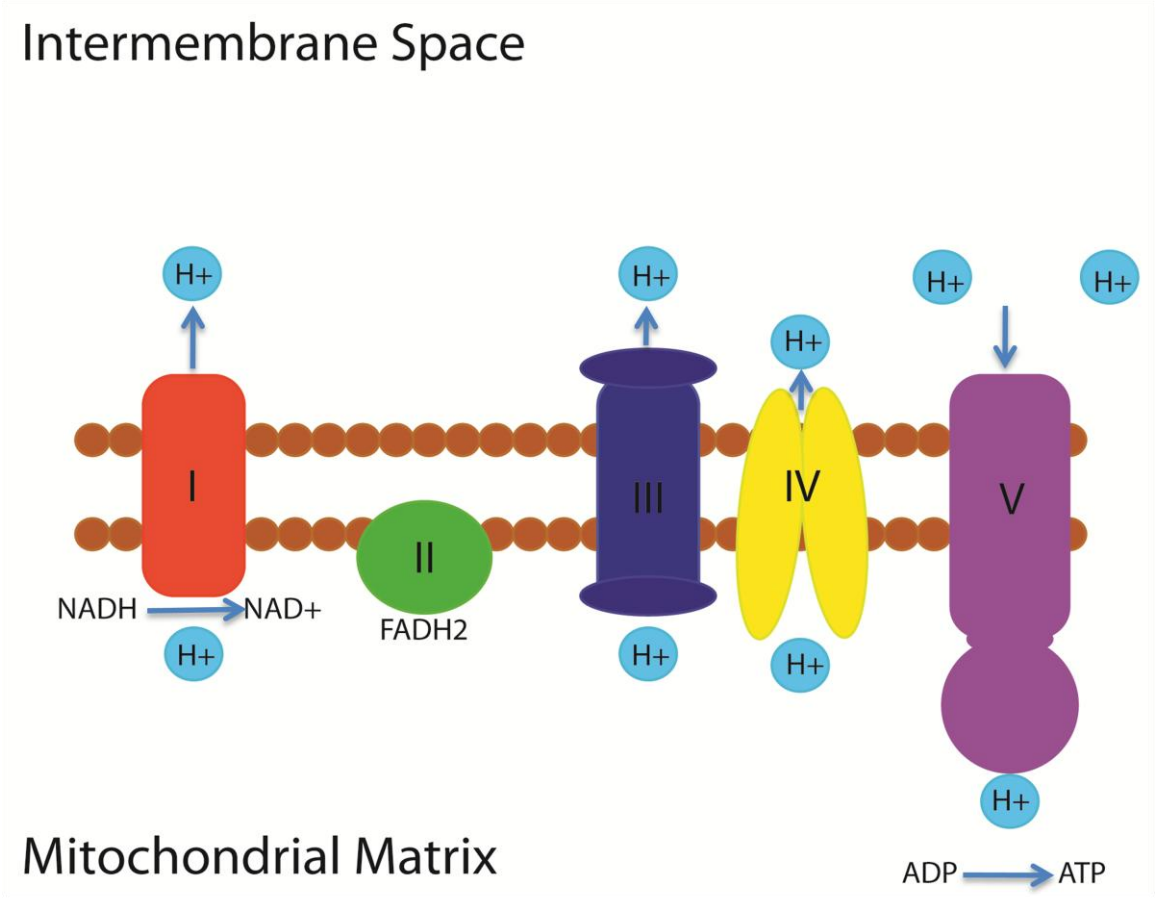

Figure 5: The Electron Transport Chain
The electron transport chain is located in the inner membrane of the mitochondria and is involved in oxidative phosphorylation. The subunits function to create a proton gradient across the inner membrane which facilitates the production of ATP. This study showed that OKL38 potentially interacts with subunits I, III, IV, and V.

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EDUCATION

The Pennsylvania State University: Schreyer Honors College — University Park, PA

Dean's List: Fall 2008, Fall 2010 — Expected Graduation: May 2011

- ***The Eberly College of Science***
  Bachelor of Science: Biology, Vertebrate Physiology Option

  Minor: Health Policy Administration

  Relevant courses: Population Genetics, Molecular/Cell Biology, Function and Development of Organisms, Medical Embryology, Mammalian Physiology

RESEARCH

Undergraduate Research Under Dr. Yanming Wang — *Fall 2008-Present*

- Epigenetic histone modification in regards to cell differentiation and cancer
- Inducible protein expression of OKL38 in the MCF-7 breast cancer cell line and siRNA knockdown experiments to test levels of p53, p21 and OKL38 expression
- Phage-display analysis to examine the role of OKL-38 in apoptosis; found evidence that OKL-38 interacts with all five subunits of the electron transport chain in the mitochondria; flow cytometry to look at the reactive oxygen species present in cancer cells after inducible OKL-38 expression