DEVELOPING A SCREENING PROCESS FOR EARLY DETECTION OF MELANOMA

by

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A THESIS

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Dr. Philip Washbourne

This thesis explores the possibility of detecting early signs of potentially life threatening melanoma through a simple blood test before the cancer reaches an untreatably fatal stage. There is promising data to support that such an assay is possible to determine whether a patient may have metastatic melanoma. This early detection is possible because the body appears to synthesize antibodies against transient receptor potential melastatin 1 (TRPM1), when under the stress of metastatic melanoma. We can easily synthesize the portion of TRPM1 peptide that the human antibody recognizes, and therefore measure whether or not the patient has these critical antibodies in their blood serum. The presence of these antibodies is a red flag and can hopefully lead to immediate examination from a doctor who may be able to stop the disease from progressing further. This assay can hopefully save many lives.

Acknowledgements

I would like to thank Professors Washbourne, Mossberg, Morgans, and Peixoto for helping me complete this process. This project would not have happened at all without the patience and guidance of Dr. Catherine Morgans, in whose lab I worked last summer at OHSU. Professor Barbara Mossberg never faltered in her belief in what I could achieve, and it prompted me to put forth my best. Professor Philip Washbourne assisted in ensuring the quality, succinctness, and clarity of my thesis. Professor Michael Peixoto provided me with profound literary edits. Staci Rogers, my soccer coach, kept me sane with several hours of scheduled soccer per week. My parents, Robert and Nell Bonaparte, never failed in their support and encouragement. I would like to thank the Robert D. Clark Honors College for pushing me past what would normally be expected of a biology major, and getting me out of my comfort zone.

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Chapter 1: Introduction

Significance of Research

Cancer has plagued mankind since our inception (Bianconi et al. 2013). Cells are constantly being replaced with new ones, albeit at different rates for different cell types. While this process keeps the human body functioning, billions upon billions of mitosis stages can take their toll on a cell (Milo and Phillips, 2016). Even though the cell has many safeguards against errors while replicating itself, mistakes do happen in rare instances. These mistakes lead to mutations in the genome. While mistakes are not always malignant, they sometimes can cause harm. Mistakes often lead to predispositions to real damage. This damage comes in the form of cancers, cells that no longer have safeguards on them. Cancerous cells can arise from many different mutations, but generally replicate with impunity, will not willingly undergo apoptosis, and will crowd out neighboring cells. In 2010, one in four deaths in the United States was caused by cancer (Jemal et al. 2010). My research aims to help at least one subset of those patients get help before it is too late.

Statement of Purpose

As one of the fastest growing and most malignant skin cancer, my research endeavors to find an effective, inexpensive, and easy way to screen patients for metastatic melanoma (Bello, 2013). In metastatic melanoma, malignant melanocytes migrate to other parts of the body, potentially spreading the cancer to critical and life sustaining organs (Lu, 2009). Screening patients for this cancer requires a reliable biomarker that sets the metastatic melanoma patients apart from healthy counterparts.

One such potential biomarker is a protein, transient receptor potential melastatin 1 (TRPM1), that is normally only found in melanocytes and ON-bipolar cells in the retina (Xiong et al. 2013). The body's immune system then produces autoantibodies against TRPM1, presumably because it is out of place.

Research Question

Does screening for presence of TRPM1 autoantibodies diagnose early stages of metastatic melanoma so that the patient can be diagnosed, treated, and helped in a timely manner?

Hypothesis

I hypothesize that this research will apply to all metastatic melanoma patients, in that all patients start creating these autoantibodies after their melanocytes start replicating out of control and spreading.

Chapter 2: Background

Cancer

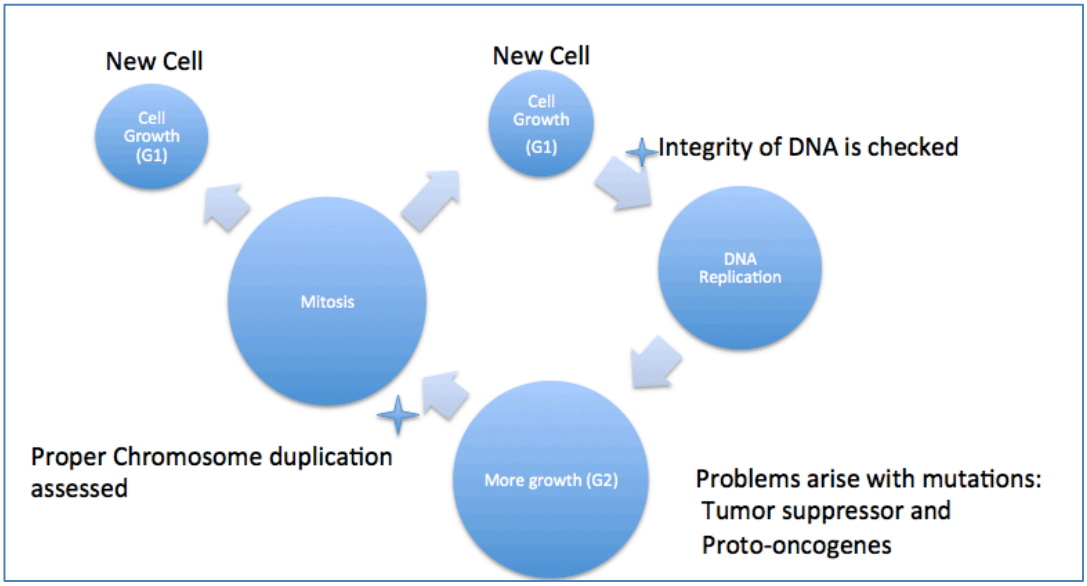

Figure 1: The cell cycle

Most cell types undergo mitosis, during which they replicate everything within them and divide into two cells. A new cell undergoes a primary growth stage (G1), at which point there is a checkpoint to make sure that the external requirements for growth have been met. After this, the cell replicates its DNA. A second growth stage follows (G2), and then another checkpoint ensues, to make sure that all of the chromosomes are duplicated correctly. Once the cell has detected that everything is in order, it pinches itself into two, and generates two independent cells, ready to either repeat the process, or remain undivided.

Mutations can occur that disrupt the checkpoints in critical ways. Certain proteins are necessary to carry out those checkpoints, and if they have lost their function, then the cell may proceed with replication without making sure that the DNA is perfectly replicated as it should be. Once DNA is replicated erroneously, it becomes

much easier for more mutations to occur in the genome. The host is in danger when these mutations eventually lead towards rapid cell proliferation and become cancerous.

Not all cells in the human body undergo many rounds of mitosis throughout a lifetime. Certain organs within a human body, however, proliferate throughout adulthood. The skin, the body's largest organ, constantly needs to regenerate in order to perform its many functions. Melanocytes are just one of the several cell types within the skin organ.

Generating Antibodies and Autoantibodies

The human immune system works by recognizing harmful intruders, marking them as dangerous, and then eliminating them from the system or blocking their function. One facet of that defensive system involves white blood cells called B-cells carrying antibodies on their surface. There are millions of unique B-cells in our blood, each coated with their own distinctive antibodies, primed to recognize a specific target. Antibody targets are called antigens. The body creates a huge amount of B-cells with randomized antibodies with the hope that it can recognize any conceivable threat. Antibodies that would recognize the self as a target are degraded (Berg 2002).

When the antibody on a B-cell happens to find its pairing with an antigen while it is circulating in the blood, it becomes activated. The B-cell begins replicating itself and producing a large amount of its unique antibodies. Some B-cells will migrate to the spleen and will wait on hold until they are needed again. The rest will release their huge stores of antibodies into the blood. These antibodies will then be able to circulate, bind, and assist in the degradation or inactivation of whatever antigen had originally activated the original B-cell. This process is why the body is so effective at fighting off a repeat

exposure to infection or disease. For example, people generally do not get chicken pox twice.

There is much mystery surrounding the immune system attacking its own cells, a process known as autoimmunity. For example, type I diabetics have had their beta cells within their pancreas destroyed, and are no longer able to produce insulin. The beginnings of this mechanism are unknown, though there are several theories on how cancer can spark autoimmune events. These include tolerance defects, inflammation, altered protein structure, cellular death mechanisms, and changes in expression levels (Zaenker et al. 2016).

In my research, I consider how cancerous melanocytes could lead to an instance of autoimmunity, although it is possible that there are simultaneous factors at play. A tolerance defect refers to the body's possible inability to delete B-cells that would recognize itself as a target. This defect remains less likely, because the body attains this autoimmunity spontaneously without a metastasizing cancer to induce it. Inflammation is a viable cause of autoimmunity, because as the melanocytes are growing out of control, it creates local sites of swelling corresponding to the proliferation of cells. This growth increases the permeability to the nearby vasculature, which would provide easier access for circulating B-cells to recognize an antigen and become active, beginning the cascade of events previously described as an immune response. Altered protein structure is a possible cause of autoimmunity, but not probable. If the same protein associated with melanocytes were mutated in such a way that it was recognized by a B-cell, then it does not make sense that the released antibodies would be able to recognize local, unaltered version of protein and become an autoimmune issue.

Another possibility of how autoimmunity develops is the way in which the body is fighting the cancer. Cells normally die through apoptosis, which is a generally a swift and clean way to eliminate a cell that is supposed to die. In killing cancer cells, the process becomes erratic and stochastic, and cell contents can spew out into places like the blood stream. Proteins that are not normally in the blood stream could then be recognized as an antigen, and be targeted by the immune system.

Finally, and similar to inflammation, altered expression levels could be the cause of autoimmune responses. With metastasizing cells, they are growing where they do not belong, and also at much higher levels than normal. This higher expression level could then be the tipping point that triggers an immune response. Metastasizing cancer cells have thousands of protein associations that could become targets for B-cells. Antibodies could be recognizing mutated proteins associated with the melanocytes, other over-expressed proteins, or other proteins that are simply out of place.

Transient Receptor Potential cation channel Subfamily M member 1

The name of the protein gives some clues to its function: transient refers to the fact that it is a protein that spans the distance between the inside and outside of a cell. It is a receptor because it is able to receive a signal and act accordingly. Upon activation it undergoes a conformational change and allows for the passage of calcium and sodium from the outside of the cell to the inside, acting as a channel. Calcium is a basic but crucially important element, because it is capable of doing so much in the cell, depending on the conditions of entry. Sodium is an important positively charged ion that will help to depolarize the cell. For the TRPM1 channels in the retina, the calcium will help propagate a certain visual pathway that takes place in low-light conditions, and

the signal will eventually form an image in the brain (Morgans et al. 2010). The function of the TRPM1 channel in the melanocytes is less understood, but it is believed to help the body produce melanin, which is the molecule that helps pigment the skin (Devi et al. 2013).

Melanoma Associated Retinopathy

Some melanoma patients develop night blindness. The problem has been coined: Melanoma Associated Retinopathy (MAR). Research has shown that this arises from autoantibodies crossing the blood-retina barrier and entering the retina. The cause of the night blindness can be localized to the ON-bipolar cells in the retina. It is hypothesized that at a certain point in the onset of melanoma, the body builds up autoantibodies against certain antigens relating to the cancer. One such antigen would be TRPM1, which is found in melanocytes and in the retina (Devi et al. 2013). The body's immune system must have recognized the TRPM1 channel where it was not supposed to be, and sent out an immune response against it (Maio, 2012). The TRPM1 autoantibodies found their way into the retina while traveling through the blood, and end up binding to the TRPM1 channel in retinal bipolar cells. By obstructing this channel in the bipolar cells, the depolarization that is necessary for the brain to interpret an image in low-light conditions is inhibited, and the patient is affected by night blindness.

Current Screening Methods for Melanoma

The current state of screening for melanoma is not very effective. At this point, doctors usually give a total-body skin examination to patients who are extremely at risk, or else they simply rely on a patient's initiative to get something checked out (Erika et

al. 2005). One must distinguish benign pigmented lesions from early melanoma. Looking out for asymmetry, border, color, diameter, and the elevation of the lesion is critical when trying to identify early melanoma. Essentially, if it is not symmetrical, flat, brown, or flush with the rest of your skin, you have reason for concern. Usually in order to diagnose the problem, the doctor would extract the lesion and wait for biopsy results. New experimental non-invasive procedures may be just as effective in diagnosing melanoma (Rigel et al. 2010). According to Rigel, the main issue in the process of helping people with melanoma is a lack of general expertise in recognizing melanoma. Health care professionals and common citizens alike are usually ill equipped to recognize what the danger signs are. An individual may be hesitant to consult a doctor about a mole that may appear to be innocuous.

Chapter 3: Experimental Procedures

Immunofluorescence

Immunofluorescence is a key method in cell biology research. It is an elegant way of harnessing the power of the relationship between antigen and antibody. An antibody sticks onto a certain antigen that it specifically recognizes. Setting up this reaction requires a reasonable amount of antigen available to be bound. The primary reaction is the addition of a primary antibody, which recognizes the antigen in question. The secondary reaction is the addition of a secondary antibody, which is often grown in some model mammal, which recognizes any antibody from the organism that made the primary antibody. This secondary antibody is also fluorescently labeled, so that it will emit a certain wavelength of light after being illuminated with light of a different (shorter) wavelength. The final step is to look at it under a microscope that can simultaneously shine the excitation wavelength and observe the emitted wavelength.

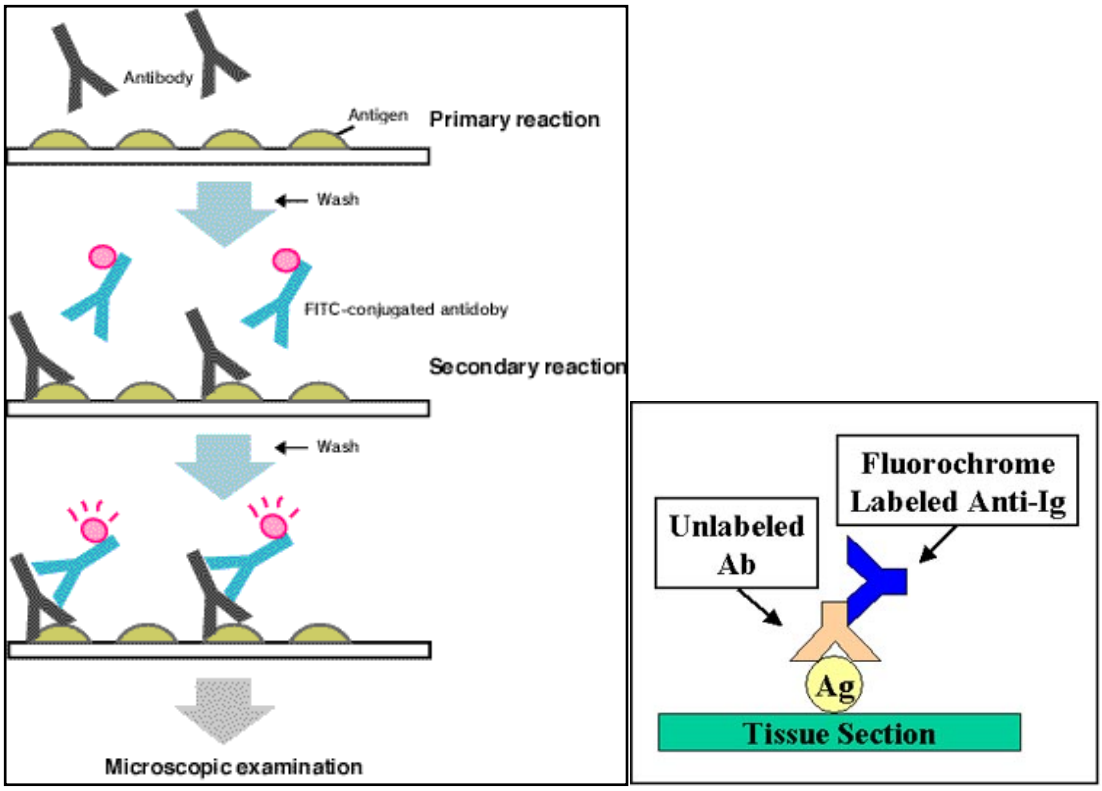

Figure 2: Immunfluorescence

http://www.slideshare.net/ali7070/immunofluorescence-39956337

Transfection

Transfection offers a second key process in cell biology research. This technique involves the introduction of DNA into a Eukaryotic cell using non-viral methods. To do this, I followed a prescribed procedure that will allow Human Embryonic Kidney 293 (HEK 293) cells to uptake a DNA plasmid containing the coding sequence of a protein of interest, transcribe it, and then translate it into the desired protein construct.

Epitope Verification

The first part of my project with Dr. Catherine Morgans at OHSU was the identification of the epitope of TRPM1 that reacted with TRPM1 autoantibodies present

in serum samples from MAR patients. Research that she had previously performed had isolated the epitope between the n-terminus and the cytoplasmic domain (Xiong et al. 2013)

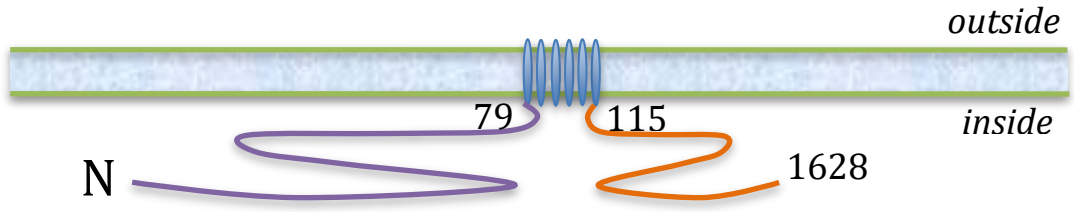

Figure 3: TRPM1 Protein

The graphic above shows the general structure of TRPM1 protein. The blue bar represents the cell membrane, the purple strand represents the stretch of peptide between its beginning and transmembrane portion, the blue elipses represent the stretch of protein that goes back and forth between the inside and outside of the cell, and the orange strand represents the section of protein between the transmembrane and the end of the protein.

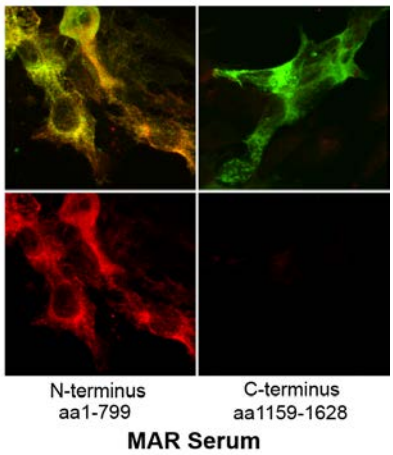

Figure 4: Epitope Narrowing

This represents the utilization of transfection and immunofluorescence techniques to glean critical information.

Dr. Catherine Morgans performed this experiment before I joined the lab. The top two squares of figure 4 illustrate when cells have been successfully transfected.

HEK cells were transfected with a plasmid containing a blueprint to construct a certain amino acid sequence and also connected with Green Fluorescent Protein (GFP). The green immunofluorescence shows that the cell's transfection was successful. If GFP is present, we can assume that the rest of the peptide was successfully transfected. The red fluorescence was a measure of whether or not the primary antibody had bound and recognized the epitope within the amino acid chain. In the case of peptide 1–799, the antibodies in MAR serum were able to recognize it, and therefore provide immunofluorescence. When HEK cells were transfected with amino acids 1159–1628, antibodies in MAR serum were unable to recognize it, which led to no immunofluorescence. This difference suggests that somewhere between amino acids 1 and 799 is a sequence of amino acids that the antibody targets, called the epitope.

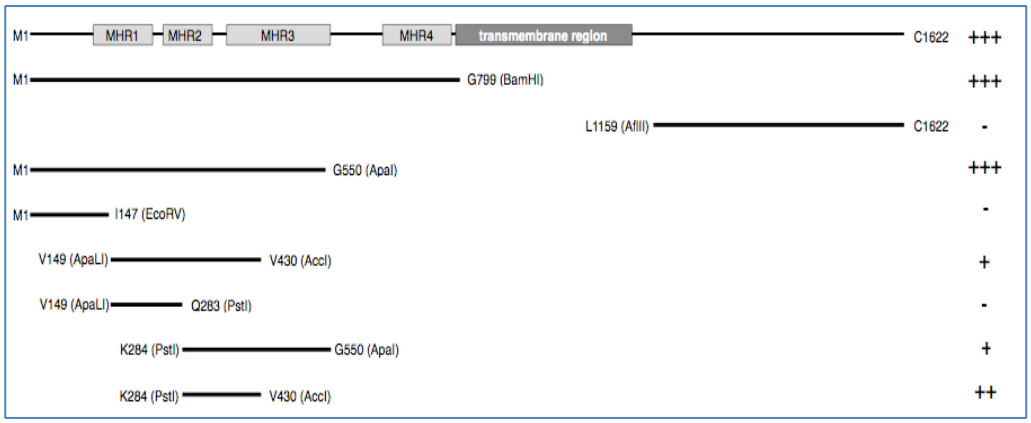

Figure 5: Further Differentiation

Dr. Morgans performed some further experiments to locate the epitope involved slicing the protein in different lengths.

The top line is another visual representation of the TRPM1 protein. M1 corresponds to the N-terminal methionine, and C1622 corresponds to the C terminus. This relationship shows the process of narrowing down which piece of protein, if

expressed, would lead to attachment of antibodies against TRPM1. These different pieces of the TRPM1 sequence were sliced up using certain restriction enzymes, which cut the DNA in specific places, depending on the enzyme used. These known smaller pieces can then be used to further narrow down where the epitope lies.

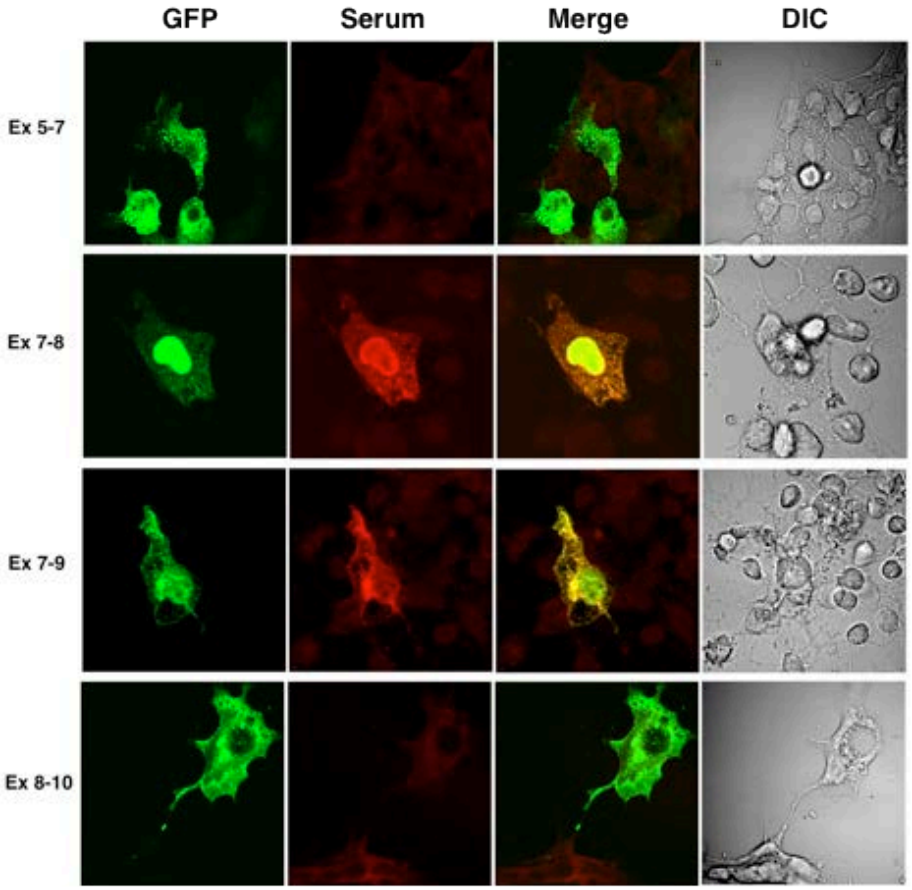

Figure 6: Further Narrowing

HEK 293 cells were transfected with plasmids encoding different regions of TRPM1 within exons 5-10 fused to GFP. The first column of images shows the GFP fluorescence of the transfected cells (green). The second column shows immunofluorescence obtained with serum from a MAR patient (red). The third column shows the merged images from the first and second columns. Areas with both red and green fluorescence appear yellow. The fourth column shows differential interference contrast (DIC) images of the cells, so that both transfected and untransfected cells can be seen.

Building on Dr. Morgans work, I was able to experimentally replicate her results, using a new MAR patient serum to identify which exons were parts of the epitope. Exons are the sections of DNA that are kept after RNA processing and will eventually be translated into protein. The whole of a gene's DNA is not expressed to

protein, because the cell trims out certain pieces that are unneeded. I expressed different exon combinations of TRPM1 in human embryonic kidney cells (HEK293) cells using transfection. Once I had cells producing these TRPM1 polypeptides, I could then probe them with the MAR patient sera that had previously been determined to contain TRPM1 autoantibodies. I confirmed that this MAR patient's serum targeted the same TRPM1 epitope as another patient's serum that the lab has previously characterized. Although I would ideally continue to test more MAR patient sera to make sure that the epitope across patients is totally conserved, time constraints made this portion of the project impossible in the present experiment. If this epitope is truly conserved, then it will be easy to synthesize peptides of the epitope and test it against patient sera to determine whether or not the patient is producing antibodies against it.

Assay Creation

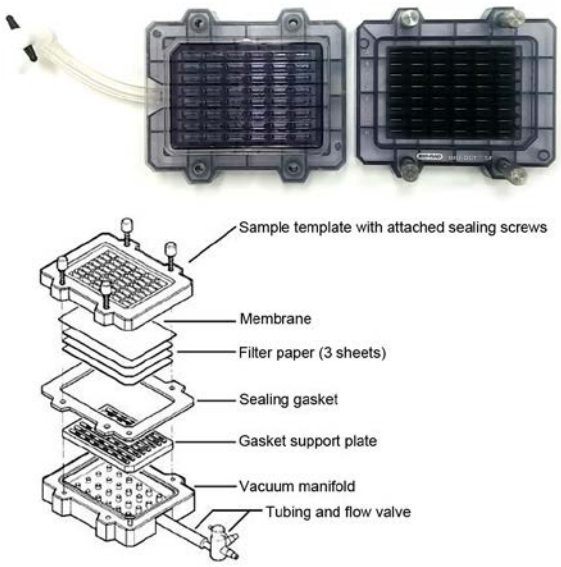

Figure 7: Slot Blot Assay

http://www.bio-protocol.org/attached/image/20150416/20150416022928_1386.jpg

Figure 7 represents the slot blot assay apparatus that I assembled. Each column had a different serum or different concentration of serum, but consistent throughout the column. Each row had a different concentration of TRPM1 peptide concentration, serially diluted down. Each slot was filled with about 200 µl of solution, which was then sucked down by a vacuum. The protein peptides stick to the nitrocellulose membrane. The slots narrowed to slits, which is where the captured proteins stayed.

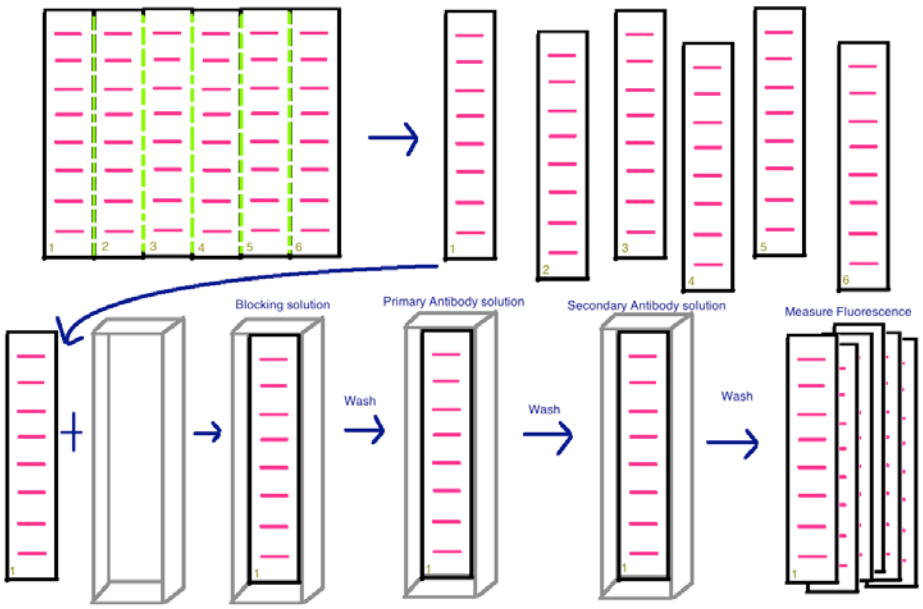

Figure 8: Assay

I separated the nitrocellulose membrane from the filter paper once it had bound my desired concentrations of peptide. I then cut the strips of the membrane into columns. These were submerged in blocking solution, which coated the rest of the membrane in a plethora of non-specific proteins. This coating was important so that all the antibodies in the serum that we are testing did not stick to the nitrocellulose and give false signals. After this blocking process, we probed it with diluted patient sera to act as primary antibodies and then probed it with secondary antibodies after another wash. We finally measured its fluorescence, which were quantified using specialized machines as signal intensity.

I had to experimentally adjust the assay to find out what parameters are ideal for the blood test. The dilutions of serum started at 1:500, then went to 1:1000, and then to 1:2000. Concentrations of the peptide along the y-axis started at 1ng/microliter, and were then halved from one well to another, in a serial dilution. I experimented with further dilutions of serum, without much success. I then tried altering the rinsing

procedure to determine whether it would be more advantageous to use Phosphate Buffered Saline (PBS) or Bovine Serum Albumin (BSA). PBS is almost analogous to using water as a rinse, and BSA is used as a blocking solution. A blocking solution would be necessary to make sure that there is no unnecessary binding to the nitrocellulose that our synthesized peptide has stuck to. The use of a blocking solution before probing our strips of nitrocellulose before adding primary antibodies made it unnecessary to add BSA at the same time as we were adding TRPM1 peptide. To do so could prevent the desired peptide from binding in the first place.

Chapter 4: Results

The results of my experiments are summarized in figures 9–11. I found that the blood test is able to differentiate between normal patients and those with metastatic melanoma.

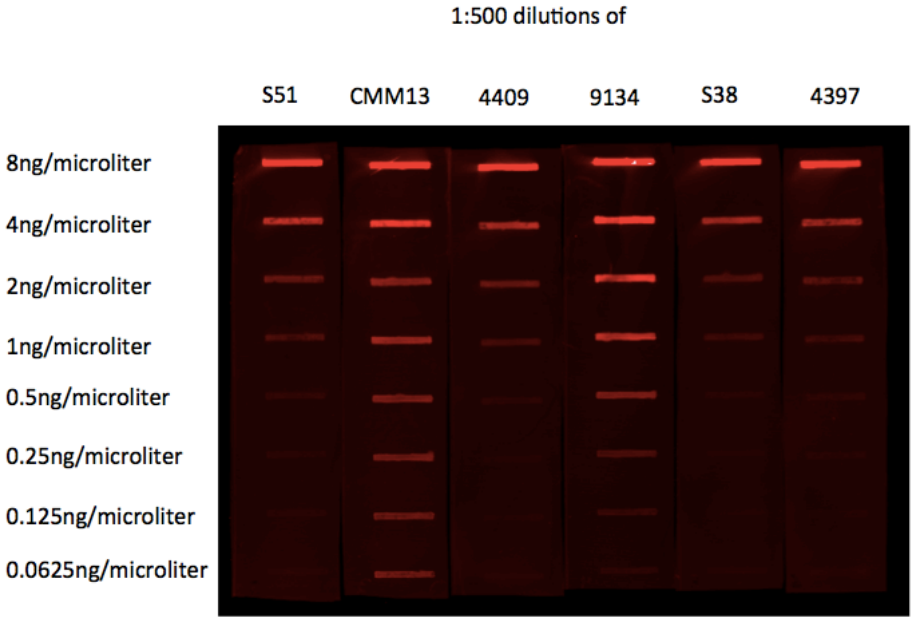

Figure 9: Final Slot Blot

This image was an example of the final slot blot test that I ran. I used six different sera, as shown above. S51, 4409, S38, and 4397 were known negatives (i.e. sera from healthy subjects); CMM13 was a patient with metastatic melanoma, and 9134 was a patient with melanoma associated retinopathy. Merely looking at this blot, columns CMM13 and 9134 clearly stand out, but it is also useful to look at a graphical representation of the data.

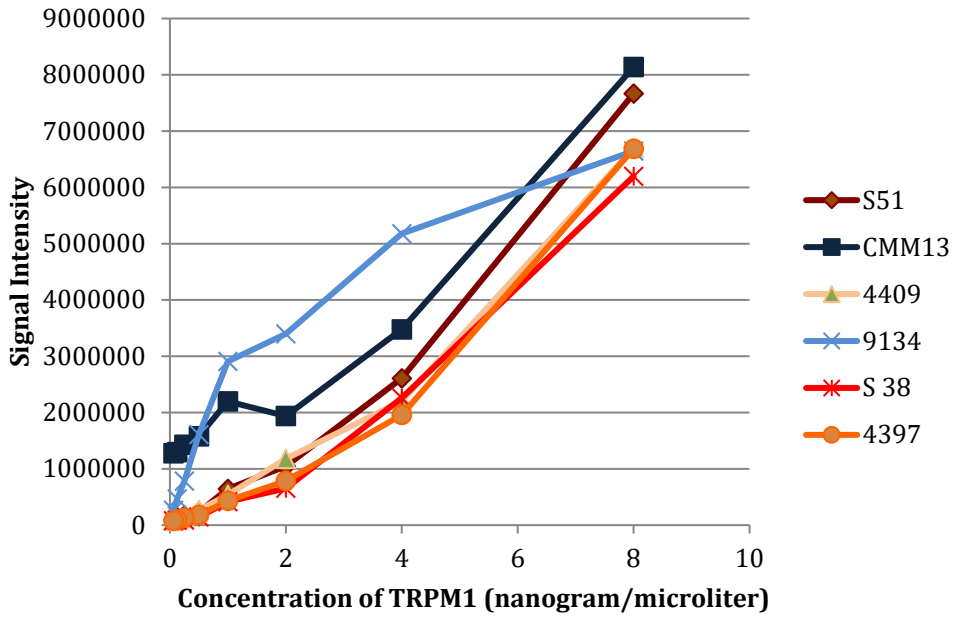

Figure 10: Graphical Representation of Slot Blot

On the x-axis is the concentration of the TRPM1 peptide. The y-axis is the signal intensity. The different lines represent the different sera that I used. At concentrations higher than 2 ng/µl of TRPM1 peptide, the signal loses specificity, and there is little difference between the negative and positive sera. At concentrations lower than 0.25 ng/µl, there is hardly enough peptide to elicit a signal from the sera at the dilution used (1:500). Between 0.25 ng/microliter and 2 ng/microliter TRPM1 polypeptide was the critical area that allowed discrimination between positive and negative sera.

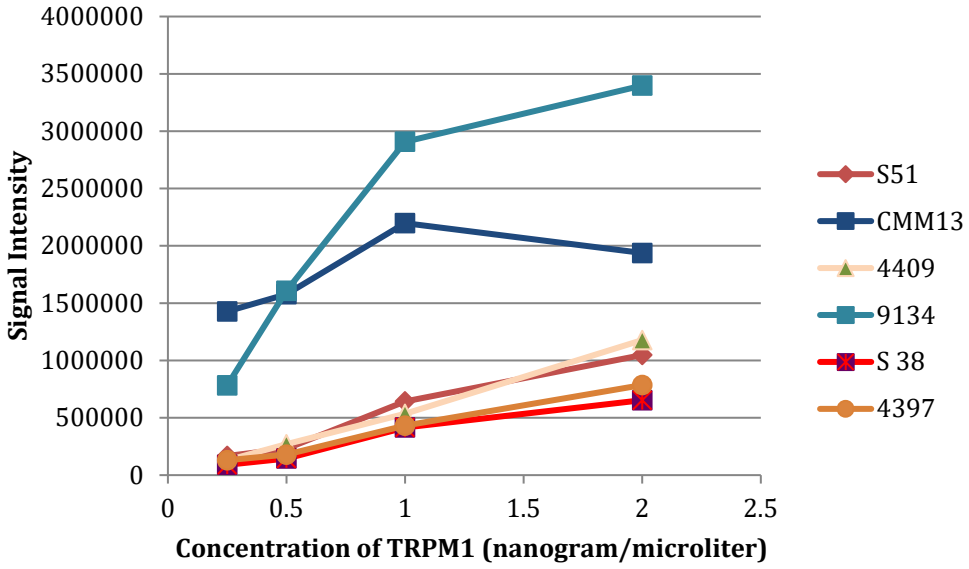

Figure 11: Blow up of Important Information

This graph demonstrates a stark contrast between the signal intensities from sera that had melanoma, and those that did not. Serum 9134 had metastatic melanoma and reported vision problems, and CMM13 had metastatic melanoma, but had no reported vision problems. The rest of the sera did not have any melanoma.

Chapter 5: Discussion

I anticipate that this research may apply to all metastatic melanoma patients, in that all patients could be creating these autoantibodies after their melanocytes start replicating out of control. I have obtained promising results, but the only way to refute or build upon them is to perform more research. If I had access to more sera of people who do not have melanoma and also from patients who are at known stages of melanoma progression, I could get a much clearer idea of how viable my research is, on a clinical level. It is crucial to establish at what stage of the cancer the autoantibodies are being produced, or what percentage of patients even produce these autoantibodies.

Connecting melanoma to certain autoantibodies arose from a rare instance. Problems with night blindness only occur in a subset of patients with metastatic melanoma when the blood-retinal barrier is compromised, allowing a small amount of leakage of the autoantibodies into the retina. It is very rare for someone to come in with issues of night blindness and have it lead to a diagnosis of melanoma, but it has happened before (Dalal et al. 2013). This technique may be able to help diagnose melanoma before situations get dire.

It is my hope that this simple blood test will provide a patient with information about the risk of melanoma. A small blood sample is considerably less invasive than a full-body examination. If the blood sample comes back positive for the autoantibodies, then the patient and doctor can work together to nip the problem in the bud, before it gets considerably more life threatening or fatal.

There are five main stages of categorizing melanoma, with many sub-stages in between. Stage 0 is just on the surface of the skin, and is easily surgically removed.

Stages I–II are still in the skin, have grown wider and deeper, yet can still be surgically removed without much worry about it spreading. It becomes more complicated in stage III, when the cancer has spread to a lymph node, which is usually responsible for cleaning out normal waste in the body. From there, it is generally easier for the cancer to spread to other organs of the body, and become classified as stage IV. When treating stage III or stage IV melanoma, it is more common to turn to radiation, immunotherapy, targeted therapy, or chemotherapy. The real danger of melanoma comes from the metastization of the cancer, spreading from the outer layer of the skin to inner layers, to the lymph nodes, and to organs that are necessary to sustain life.

This detection of TRPM1 autoantibodies will have prognostic value to the patient. Hopefully, this method could catch the problem at stage III, where it is more easily treatable, before it goes to a more dangerous stage IV. It is also possible that the presence of antibodies against TRPM1 is an indication that the body is putting up a fight against the cancer, and that the patient has a high chance of survival.

Immunotherapy is a burgeoning field in the fight against melanoma and cancer in general. The treatment essentially aims to help the body fight its own battle against the cancer by boosting the immune system, or helping the body recognize the cancer as something to be destroyed. It would be interesting to research how TRPM1 autoantibodies could be incorporated into the therapeutic approach. Of course, there would always be the dangerous potential side effect that the antibodies would cross the blood brain barrier and interfere with the function of the TRPM1 protein in the retina.

Moving forward, there is real promise in the further study of the role that TRPM1 autoantibodies play in patients with melanoma. Using my assay, it will be easy

to gain a greater understanding of how these antibodies are playing a role in patients at different stages of their cancer. With any luck, the autoantibodies will be at significant levels and can be detected before the cancer has had a chance to spread to critical organs, and lives can be saved.

Glossary

Apoptosis: The process of controlled suicide that a cell undergoes if it is functioning, and has reached a point at which it should die.

Amino acid: The building blocks of a protein. There are 21 unique ones. Each one has an amine group (NH_2), a carboxyl group (COOH), and a side chain. These side chains are what make each amino acid unique. The first amino acid has a free amine group, and then a peptide bond forms between its carboxyl group and the next amino acid's amine group. The amino acids daisy chain along to form a polypeptide.

Antibody: A unit of immune response whose job is to tag certain sequences or cells. Different antibodies perform many different functions.

Antigen: The sequence of amino acids that an antibody recognizes as a target.

Assay: A biological test, where something is being tested. There is often a negative control, a positive control, and one to several experimental procedures.

Autoantibody: A unit of immune response that recognizes some part of its own host as something that needs to be deactivated or destroyed.

Blood brain barrier: A highly selective permeability barrier that separates the blood from the central nervous system (brain). Antibodies cannot usually get through, but may get through in small concentrations.

Blood retinal barrier: Very similar to the blood brain barrier, except that this barrier is specifically at the choroid capillaries to retina connection.

Capillary: A very small blood vessel that facilitates the exchange of nutrients, waste, and some other substances with the tissue surrounding it.

Cancer: Mutations build up in the DNA, and cause problems to arise. Either proto-oncogenes are affected, or tumor suppressor genes. These changes lead to rapid proliferation of cells, which will grow out of their usual spaces, eventually taking over other parts of the body. As the cancer spreads, it infects other cells, and normal functions start to fail.

Channel protein: This is a protein that creates a specialized opening in a cell membrane that will let a certain molecule in or out, depending on current conditions inside or outside the cell. TRPM1 is a channel protein that is calcium permeable.

Chromosomal DNA: The DNA that makes up the genome of an organism, and is conserved throughout all the organism's cells. Chromosomes are huge strings of DNA, each containing hundreds of genes. Humans have twenty-three pairs of chromosomes.

Choroid: This is the vascular layer of the eye, providing oxygen and nourishment to the outer layers of the retina.

C-Terminus: The last amino acid in a protein, as defined by its open carboxyl Group (COOH).

Depolarization: refers to how a cell propagates a signal. Cells are normally held in a state of hyperpolarization, lower in voltage than the surrounding interstitial fluid. When cells intake positive ions, it is voltage potential increases, closer to that of the surrounding fluid, therefore depolarizing.

Differential interface contrast: Microscopy technique to view contrast in in transparent samples. Good and common for visualizing cells.

Dilute: To lessen the concentration of something, usually done by adding water to the solution.

DNA: An essential part of biology. It is essentially the coding that dictates much of who a person is. A person's genetic code refers to their whole sequence of DNA. Everyone has a unique genetic code. Even twins have very slight variations.

Enzyme: Is a type of protein. They generally accept a substrate, undergo some shape change, release a product, and undergo another shape change so that it can perform the process again and again.

Epitope: The sequence that an antibody recognizes and latches onto.

Eukaryotic cells: Cells that have a nucleus and organelles encased in membranes.

Genome: The biological information of heredity that is passed down from organism to organism. Each genome is unique, as it is the specific order of nucleotides making up the DNA of an organism. Genomes between siblings will look much more similar than genomes between distant strangers, and even more so if comparing across species.

HEK293 cells: Human Embryonic kidney cells, are a specific cell line developed to grow in tissue culture. These cells are relatively easy to grow, and you can experiment on them effectively because they grow well and are transfectable.

Interstitial fluid: The liquid that makes up the space in between cells.

Metastasis: When the primary growth of cancer expands, and a second site within the patient develops the cancer.

Melanocytes: A class of cells found in the epidermis (skin).

Melanoma: Refers to skin cancer that develops in Melanocytes.

Nucleotides: the building blocks of DNA. They are the individual code pieces. For example: the 0s and 1s of computer code.

N-Terminus: The beginning of a protein, as defined by its open amine group (NH_2)

Nucleus: The compartment within a cell that houses DNA.

ON-bipolar cells: A class of cell types found in the retina (eye).

Permeability: The ability of a molecule to pass through a membrane. A high permeability refers to easy access.

Plasmid: A section of DNA that can be replicated independently and separately from the chromosomal DNA. Usually found in bacteria. Usually small and only contain a few genes in them.

Polypeptide: This is a chain of amino acids. It folds in on itself, depending on the properties of the side chains that make up its amino acids. This folded mass can either be autonomous, or may link up with another folded polypeptide to form a fully formed protein.

Protein: The little highly specialized 'machines' that carry out most functions within the body. RNA is translated into a protein.

Proto-oncogenes: These are genes that code for processes that already help the cell divide and grow. When mutated, it is possible that they become more potent, and lead to more growth/division than normal.

Restriction enzyme: Cuts DNA at a certain site along the nucleotide sequence, depending on which enzyme it is. There are varying levels of specificity.

Serum: Refers to the parts of the blood that are not red or white blood cells, clotting factors, or fibrinogens. This essentially refers to most proteins, electrolytes, antibodies, antigens, and hormones. We mostly cared about the antibodies present.

Substrate: Refers to a molecule that acts as an input in a biological reaction.

Transcription: In biology, this term is used to describe how DNA is converted into RNA.

Transfection: Introducing nucleotides into Eukaryotic cells via non-viral methods.

Translation: In biology, this term is used to describe how RNA is converted into protein.

TRPM1: Transient receptor potential melastatin 1, essentially it is an ion-gated protein channel found in melanocytes and ON-bipolar cells.

Tumor suppressor genes: These are genes that code for processes that generally keep growth in check, proof-reads DNA sequences, or signals cell death in times of emergency. When these genes are rendered useless through certain mutations, it becomes easier for cells to grow out of control.

Vasculature: Refers to the organization and distribution of blood vessels throughout the body.

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