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The Maintenance of Tumor HLA-A2 Expression In Differing Culture Environments

Jillian Harmon
Carroll College, Helena, MT

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Department of Biology.

Director: Brandon A. Thedford 3/31/14

Reader: [signature] 3-31-14

Reader: [signature] 3/31/14

THE MAINTENANCE OF TUMOR HLA-A2 EXPRESSION IN DIFFERING CULTURE ENVIRONMENTS

Jillian M. Harmon

Department of Biology
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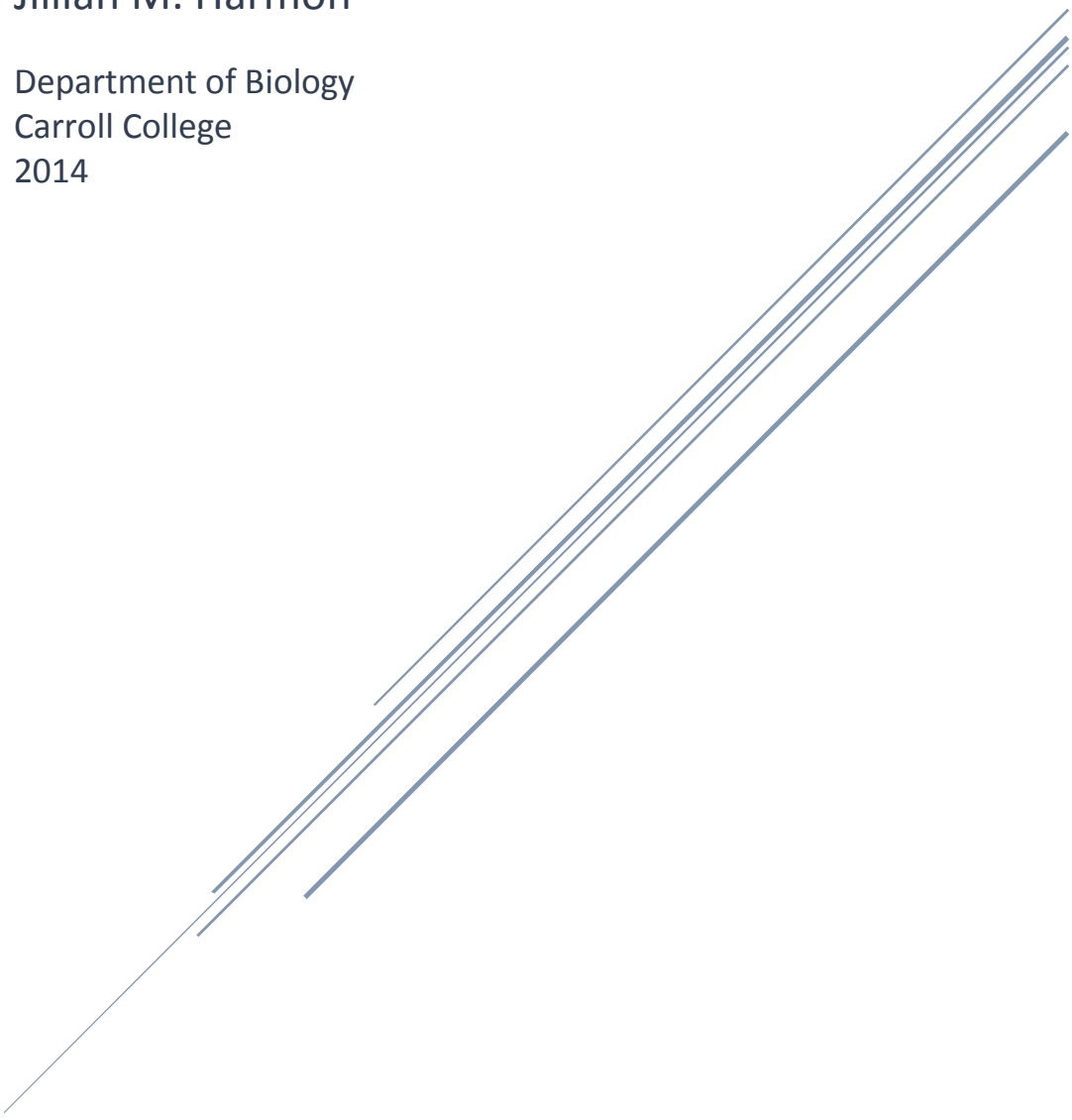


Abstract

The Maintenance of Tumor HLA-A2 Expression in Differing Culture Environments

Adoptive T cell immunotherapy is an emerging field in the research of cancer treatment. Yet, despite the scientific expansion of knowledge in this area, obstacles have arisen that hinder the rapid progression of this field. Tumor cells have developed evasion mechanisms that impede effective recognition of cancerous cells by the immune system, specifically by cytotoxic T cells. The downregulation of human leukocyte antigen A2 displayed on surface major histocompatibility complex class I (MHC I) is such a tumor evasion technique. The objective of this study was to assess the maintenance of MHC I surface expression on *ex vivo* tumors in different cell culture media. Three tumor cell lines (one p53 gene knockout and two *ex vivo*) were cultured in Dulbecco's Modified Eagle Medium (DMEM) and Roswell Park Memorial Institute (RPMI) medium for eight weeks. Twice per week, the cell lines were sampled, stained, and analyzed through flow cytometry for the amount of HLA-A2 surface expression. Despite the significant amount of fluctuation and general downregulation of HLA-A2 in all three tested cell lines, the DMEM medium maintained HLA-A2 expression in a more consistent manner amongst one of the cell lines. In the future, more research should be performed to further investigate the reasons behind DMEM’s effect, as well as the mechanisms of HLA-A2 downregulation.

Introduction:

Adoptive T cell immunotherapy is a mechanism of fighting cancer through enhancing the body's natural immune response to cancerous tissue. With the progress of adoptive T cell immunotherapy, research is pushing to new heights in the development of nontoxic treatments for cancer (June, 2007). Nevertheless, the strategies of immune defense pose a large hurdle in the progression of further laboratory research (Ahmad et al., 2004). An increasingly common tumor cell defense mechanism against specific host immunity is the downregulation of tumor associated antigens (Romero et al. 2005). Human leukocyte antigens (HLA) are linear epitopes presented by class I major histocompatibility complexes (MHC I) on the surfaces of tumor cells. The presentation of HLA in the MHC I complex plays an integral role in the interaction of cytotoxic T lymphocytes and natural killer cells with tumor cells (Hicklin et al., 1999). These MHC I complexes present mutated endogenous proteins and/or protein fragments as a distress signal, targeting the cells on which they are expressed for destruction by cytotoxic T lymphocytes (Theobald et al. 1995). The downregulation of human leukocyte antigens on presenting cells is a key mechanism in host immunity evasion by cancerous tissue (Qiao et al., 2009).

Adoptive T cell immunotherapy operates on the premise that highly specified T cells will be reactive and effective against general tumor antigens, therefore demonstrating them to be effective on a wide range of cancerous tissues (Theobald et al., 1995). Human p53 is a nuclear protein and transcription factor encoded by the TP53 gene

that, when expressed, plays a key role in halting a malfunctioning cell cycle and stimulating apoptosis, thereby facilitating tumor suppression. It has been found that at least fifty percent of cancerous cells have mutated p53 proteins (Zhua et al., 1999). When healthy cells begin to malfunction or become malignant, normal cellular proteins that are normally found at low levels can be overexpressed. The same occurs with the p53 protein (Zhua et al., 1999). When p53 is mutated, it is packaged into an MHC I complex in the endoplasmic reticulum as an antigenic epitope and presented at the cell's surface (Zhua et al., 1999). Therefore, the human p53 epitope is an ideal therapy target because it is mutated and hyper-expressed in most cancerous cells that should be targeted for destruction (Davis et al., 2010). Theobald et al. (1995) transduced a human T cell antigen specific against mutated p53 into a murine model to successfully test this theory. However, there is an endogenous level of baseline p53 expression by healthy cells throughout the mammalian body, particularly in the spleen and thymus (Theobald et al., 1995). Host T cells are therefore somewhat desensitized to this endogenous level of p53 (Theobald et al., 1995). The same principle can be applied to the recognition of HLA as a factor in T cell specificity and recognition (Karre et al., 1986; Sherman et al., 1992). As proposed by Lampen and Hall (2011), defects in and/or loss of MHC I holding the mutated p53 epitope directly affects the subsequent effectiveness of transduced T cells against tumor cells utilized in laboratory research.

Prior to this project, p53 knockout (p53-/- $A2K^b$) mouse embryonic fibroblasts were isolated from p53 knockout mice and were immortalized into a culturally viable cell line (Huber et al., 2005). The mouse embryonic fibroblasts from the p53 knockout mice were transduced with a wildtype mouse p53 gene. Additional p53 genes were also

transduced, containing hotspot mutations mut7 p53 R270C, mut4 p53 R172H, and the human p53 sequence 264-272 (Huber et al., 2005). The human 264-272 peptide coding sequence has high affinity for MHC I, and elicits a peptide specific response from T cell receptors (Theobalk et al., 1995). A hotspot mutation refers to a region of DNA sequence that is particularly susceptible to mutation or change. To immortalize the cells, the embryonic fibroblasts were transduced with a helper plasmid (pCMV.VSV-G) and two retroviral constructs (pBabe-hygro-E1A/ - H-ras). The cells were then selected with Hygromycin B and transduced with the wildtype mouse p53 gene and point mutation human p53 epitope (position 270 of the gene A arginine (R) is replaced by cysteine (C)). All tumor cell lines were prepared by the Theobald working group of the Johannes Gutenberg University Medical School.

The HLA expression of cultured tumor cells needs to remain consistent for proper T cell assay application. The environments in which the tumor cells are cultured therefore play a key role in experimental cell line health. Cell culture media provides sources of energy and other compounds that facilitate cell cycle regulation (Meenakshi, 2013). A basic cell medium contains amino acids, vitamins, inorganic salts, and glucose (Meenakshi, 2013). Some media may be supplemented with serum to provide cellular growth factors, hormones, and additives that aid in cellular attachment. Culture media also maintains pH, partial pressure of oxygen, and partial pressure of carbon dioxide in the extracellular environment through natural or artificial buffering systems (Meenakshi, 2013; Morgan et al., 1950). A natural medium is composed totally of biological extracts. Artificial media, such as Dulbecco's Modified Eagle Medium (DMEM) and Roswell Park Memorial Institute (RPMI) medium, add nutrients to maintain and aid cell growth to the

optimum potential. Particular carbohydrates, salts, serum proteins, buffering systems, and organic/inorganic nutrients may be added to tailor a medium to an individual cell line (Morgan et al., 1950). Media can be modified to better support long term cellular survival, specialized cellular function, and immortalized cell lines (Morgan et al., 1950). As a result, the medium in which cells are cultured can significantly impact cellular processes, including antigen expression.

The objective of this project was to culture a p53 knockout tumor cell line, as well as two *ex vivo* tumor cell lines, in a variety of media to analyze for an environmental impact on HLA-A2 expression. Prior to this study, it was observed that tumor cell health appeared to be better in DMEM medium, as opposed to RPMI medium. Therefore, it was hypothesized that HLA-A2 expression by immortalized tumor cells is maintained to a better degree in DMEM in comparison to RPMI medium.

Methods and Materials:

The transduced p53 knockout cell line and two *ex vivo* tumor cell lines were cultured in parallel in either DMEM or RPMI for eight weeks. Antigenic staining for HLA-A2 and flow cytometry were utilized twice a week to measure the mean fluorescence staining intensity, as well as the percent of positive HLA-A2 surface expression in respect to the sample's total cell population. The percent of positive expression and mean fluorescence intensity were tracked throughout the eight week test period.

Culturing cells in test media:

The three tumor cell lines utilized for this project were maintained in either RPMI 1640 (Lonza, Bio Whittaker) supplemented with 10% fetal calf serum (FCS) (PAA), 100 U/ µg/ mL penicillin, 1% L-glutamine (Gibco) and, 100 µg/ mL streptomycin (Pen/Strep) (Gibco) or Dulbecco's modified Eagle's medium (DMEM, Lonza, BioWhittaker) supplemented with 1% L-glutamine, 10% FCS, 1% NEAA (Lonza, BioWhittaker), 100 U/ µg/ mL Pen/Strep, and 10 mM HEPES (Lonza, Bio Whittaker) for three weeks previous to collection of data. The cells were split into Corning cell culture flasks (T75), with vented caps, at a density of 0.6×10^6 cells per 13 mL medium. This splitting routine was performed on Monday and Friday of every week. To begin the culturing process in RPMI medium, 0.2×10^6 cells were split into a Corning T25 flask with 8 mL RPMI medium. As before, this cell splitting was performed on Monday and Friday of the same week. The cells were collected via trypsinization (1X Trypsin-EDTA 0.25%), centrifuged down at 1500 rpm (approximately 500 xg) for 5 min, resuspended in 5 mL RPMI, and

transferred into a Corning T75 flask. To bring the final volume in the flask up to 13 mL, 8 mL of fresh culturing media was added to each flask. The cells were then cultured over the weekend.

Cell splitting:

The cells were split into Corning T75 flasks with vented caps for further culture throughout the experiment. The cells were observed under a microscope prior to handling to assess cell health and density. If the cells were 35-40% confluent, they were subsequently split, and aliquots of cell stock were taken for HLA-A2 staining and flow cytometry analysis. The cells were trypsinized with 1.5 mL trypsin (0.25% Trypsin-EDTA solution) and incubated at 37 °C for three minutes. The bottom of each flask was tapped to ensure the detachment of all cells. The flasks were then washed with 8.5 ml of either DMEM or RPMI, and the total 10 mL volume was transferred into 50 mL Falcon centrifuge tubes. From these cell stock solutions, 20 μL were diluted into 80 μL of Trypan Blue staining solution. The dilutions were then counted on a hemocytometer to determine the cellular concentration in the stock solution. Cells were reseeded into Corning T75 flasks at a concentration of 0.6×10^6 cells/flask in 13 mL of fresh medium. The flasks were then incubated for three to four days at 37 °C and 5% CO_2.

Cell Staining:

Fluorescence activated cell sorting (FACS) is used to analyze and quantify cell surface molecule expression. It is also used to discriminate between different cell populations in a tissue homogenate. In this study, FACS was utilized to determine the

amount of HLA-A2 expression on the tested tumor cells. From the cell stock solutions (same trypsinized stock solutions used for re-seeding the cells), 0.3×10^6 cells were aliquoted directly into FACS tubes containing 2.0 ml sterile PBS (phosphate buffered saline) solution. One aliquot was allotted for an isotype antibody to later be measured for the purpose of eliminate background staining noise. The isotype is a form of the staining antibody that binds to targets nonspecifically and works as a negative control. In each sample, there is an unknown percentage of cells that are positive for HLA-A2. The isotype is used to define the standard for a negative reading of HLA-A2 expression on a cell. The isotype is also used to set the flow cytometry analysis gating, defining what percentage of the cell population in the sample is positive for HLA-A2 expression and what percentage is negative for HLA-A2 expression.

The FACS tubes were organized and stained as such:

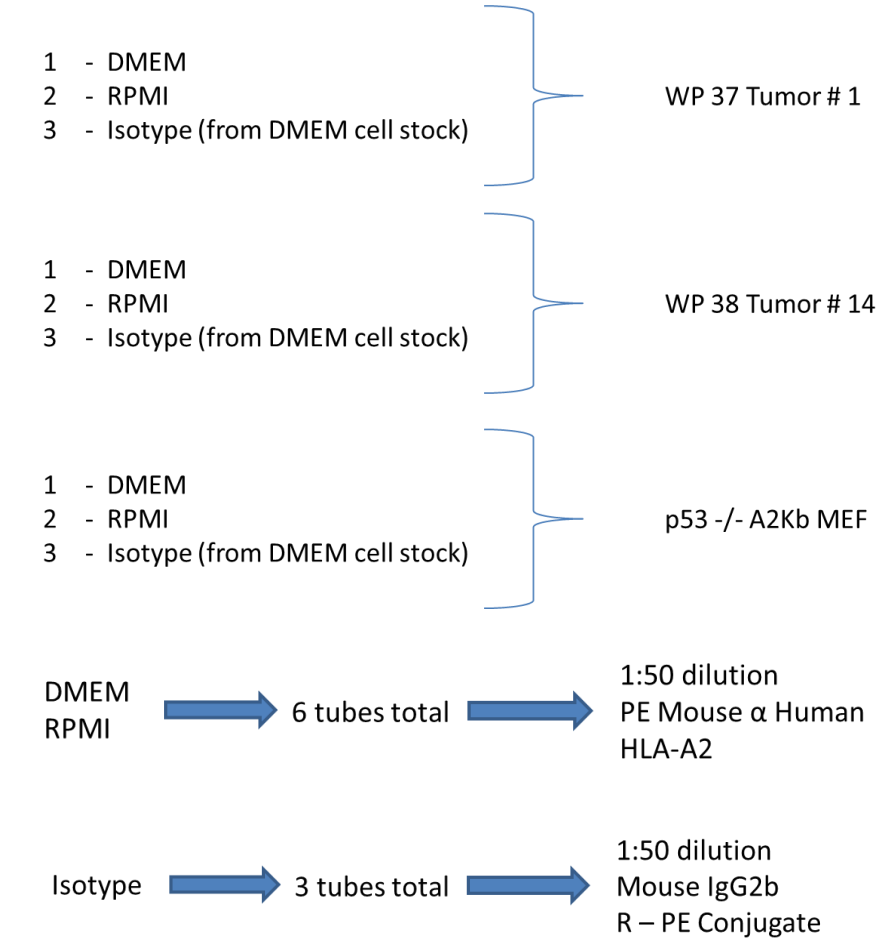


Fig. 1. Tube setup and antibody staining scheme

A total of nine tubes were used per FACS staining. The 0.3 x 10^6 cells aliquoted out of the cell stock for isotype staining was always taken from the cell stock cultured in DMEM. All FACS tubes containing the cells were washed with 2 mL of 1x PBS and centrifuged at 600 x g for 3 min. at room temperature. The excess PBS was discarded and the cell pellets were resuspended in 50 µL MACS (magnetic affinity cell sorting) buffer (0.5% BSA, 2 mM EDTA in PBS) containing 1µL of the fluorochrome-coupled antibodies (see Figure 1). Aluminum foil was used to cover the tubes, and the cells were

stained for 30 minutes at 4 °C in a dark refrigerator. The staining antibody was light sensitive, so all work with the antibody and antibody stained cells was performed in a dark sterile hood. Lastly, the samples were again washed in 2 mL 1x PBS (600 x g, 3 min, RT) and resuspended to a concentration of $0.1x10^6$ cells/100µL in 1% PFA (paraformaldehyde)/ PBS.

Flow Cytometry:

The samples were then acquired on a BD FACS Canto II. The data were exported and analyzed using FlowJo Data analysis (TreeStar Inc., Ashland). Mean fluorescence intensity (MFI) readings were calculated using the FlowJo Data software. MFI directly links the strength of the recorded fluorescence signal to the number of molecules expressed on the cell surface.

Staining Compensation:

The samples were acquired on a FACS Canto II (BD Bioscience). Automated staining compensation was performed to allow for the detection of single fluorochromes within a sample of different fluorochromes. The Automated compensation feature of the Diva Software for the FACS Canto II was used to avoid false negative signals caused by emitted spectral overlap. Single color stainings were performed with OneComp eBeads (eBioscience). One drop of OneComp eBeads was transferred in a FACS tube. Exactly 0.4 µL of the specified antibody (Figure 1) was applied to the sample, mixed, and incubated for 15-20 min at 4°C in a dark refrigerator. Samples were resuspended in 2 mL

PBS and centrifuged for 600 x g for 3 min. Lastly, samples were resuspended in 0.2 mL FACS Buffer and analyzed as single staining controls for compensation.

Results:

The HLA-A2 expression vacillated the range of 40% in the *ex vivo* cell lines. Throughout the study, a large and periodical fluctuation in the expression of HLA-A2 was observed (Figure 2). The fluctuation pattern was roughly followed by all of the tumor cell lines tested and was significantly pronounced for the p53 -/- $A2K^b$ MEF cell line cultured in DMEM (Figure 2C). There was a constant decrease in overall HLA-A2 expression in all three tumor cell lines throughout the study. The expression of HLA-A2 in the Tumor 1 cell line displayed an overall steadier level of antigen expression over the eight week period when cultured in DMEM (Figure 2A) The expression of HLA-A2 was significantly downregulated in the p53 -/- $A2K^b$ MEF cells cultured in RPMI (Figure 2C). The Tumor 14 cell line showed no preference for either medium in regard to HLA-A2 expression (Figure 2B). The same fluctuation pattern was observed in the mean fluorescence intensity (MFI) trend for each cell line (Figure 3).

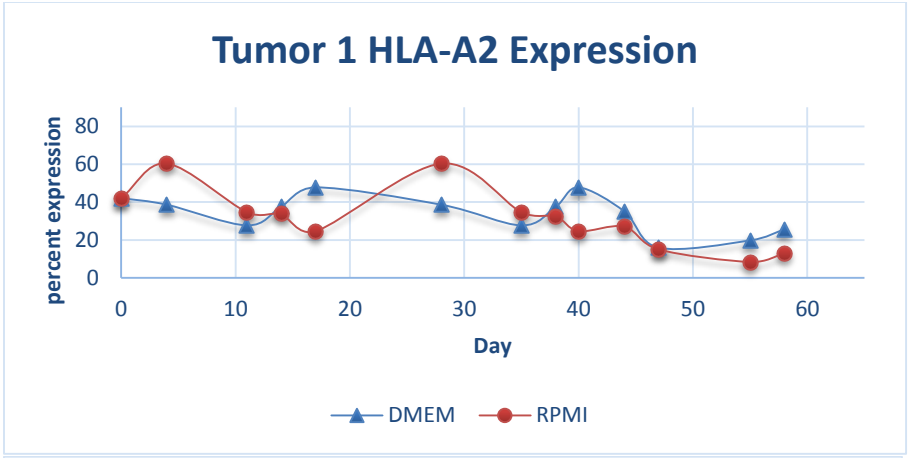
Once again, the HLA-A2 mean fluorescence intensity of the Tumor 1 cell line cultured in DMEM showed more consistency over the experimental time frame (Figure 3A). Also noteworthy, the MFI displayed by the p53 -/- $A2K^b$ MEF cells was significantly smaller than that shown by the other two cell lines tested (Figure 3C). The total fluor intensity of the p53 -/- $A2K^b$ MEF cells was approximately one third of the fluor intensity displayed by the other cell lines in both mediums. Nevertheless, the MFI was still significantly higher in the p53 -/- $A2K^b$ MEF cells cultured in DMEM. The MFI

of the Tumor 14 cell line likewise followed the trend of its percent positive HLA-A2 expression (Figure 3B).

Another notable graphical trend must be taken into account. During the stretch of time approximately between days eighteen and twenty-eight, no sampling and testing for HLA-A2 expression took place. Throughout the rest of the study, the time periods lapsing between each sampling and staining are markedly shorter. In all of the cell lines, the HLA-A2 expression was at its highest in the sample taken around day twenty-eight, just after the longer time lapse. The MFIs of all tumor cell lines follow this same data point trend on day twenty-eight.

The reasons driving HLA-A2 downregulation on tumor cells were not investigated in this study. However, was observed that the expression of HLA-A2 in the Tumor 14 cell line was independent of culture medium. The p53 knockout cell line demonstrated significantly lower HLA-A2 expression in RPMI as opposed to DMEM. This same knockout cell line exhibited noticeably lower mean fluorescence intensity in both DMEM and RPMI. Lastly, in observing the percentages of HLA-A2 expression and the subsequent MFI measurements, it was apparent that DMEM promoted steadier HLA-A2 expression over time in the Tumor 1 cell line.

A.



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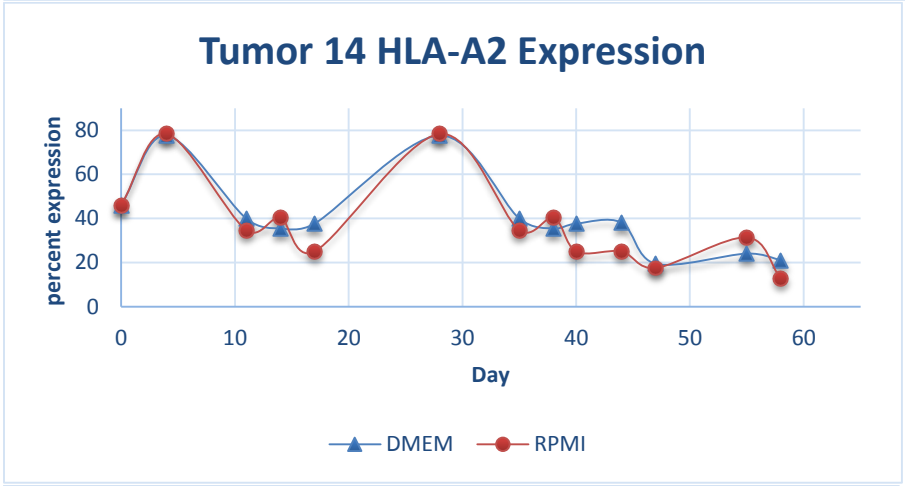

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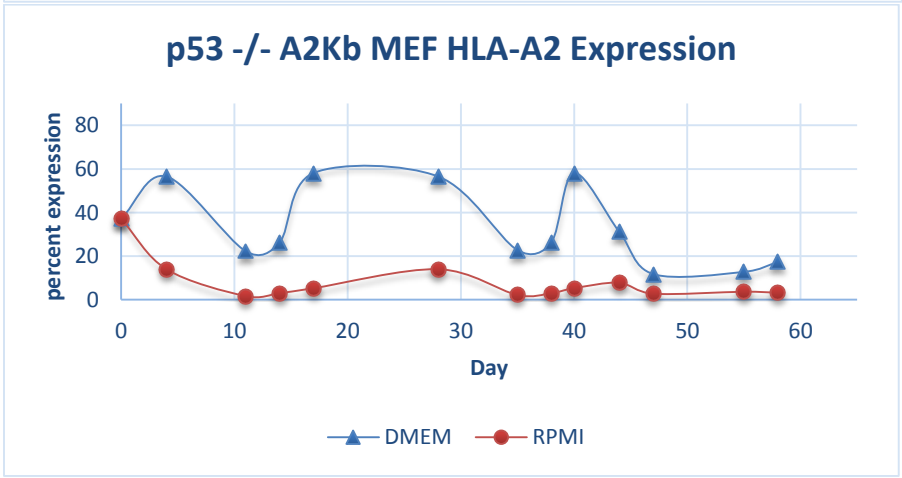
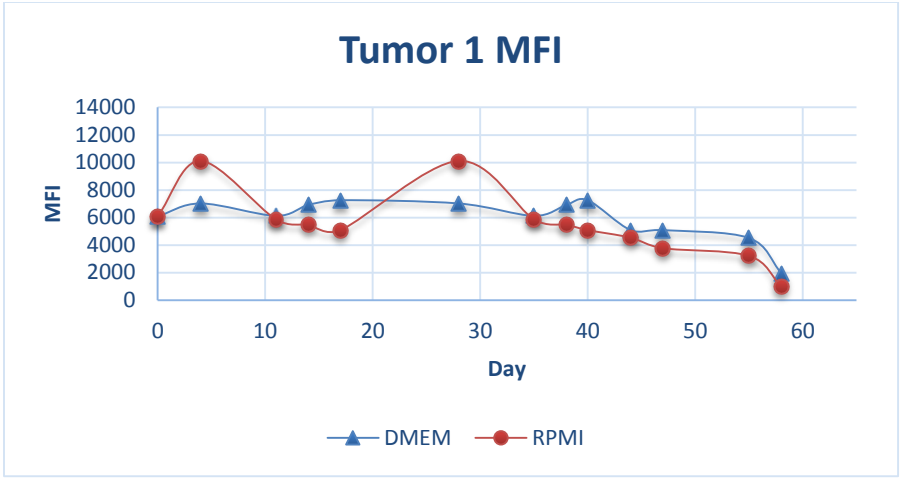


Figure 2. Percent expression of HLA-A2 on three tumor cell lines cultured in DMEM or RPMI

Graph A displays the percent of HLA-A2 expression on Tumor 1 cells cultured over the experimental time frame in either DMEM or RPMI media. Graphs B and C display the same information for the Tumor 14 and p53 -/- A2Kb MEF cells cultured over the experimental time frame.

A.



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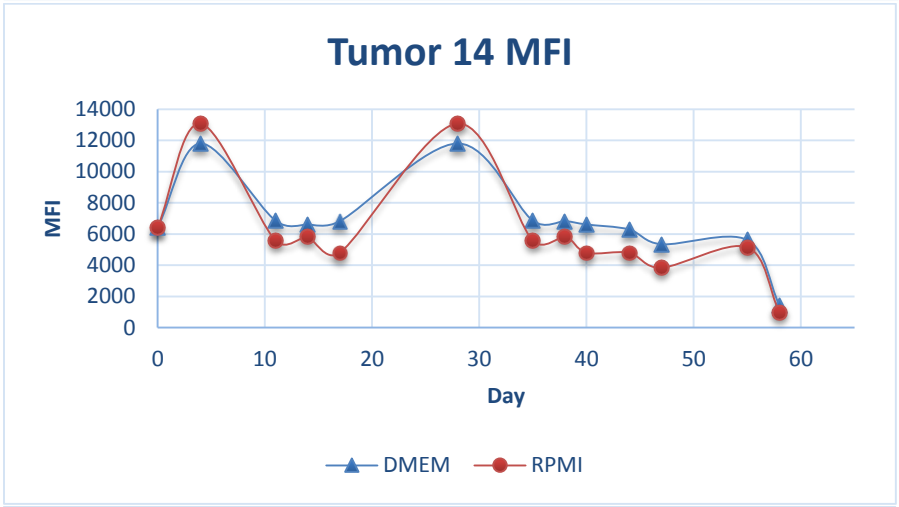

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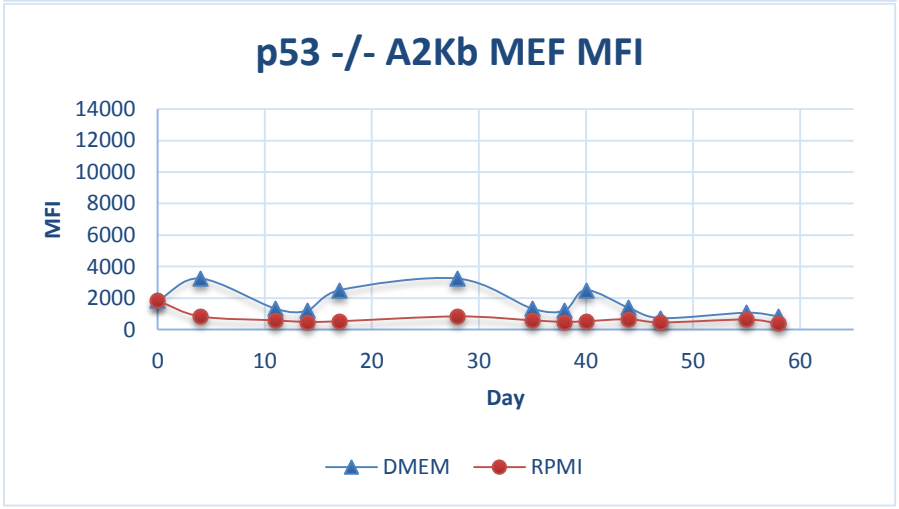

Figure 3. Mean Fluorescence Intensity of tumor cell lines cultured in DMEM or RPMI

Graph A displays the mean fluorescent intensity of HLA-A2 expressed on Tumor 14 cells cultured over the experimental time frame in either DMEM or RPMI media. Graphs B and C display the same information for the Tumor 14 and p53 -/- A2Kb MEF cells cultured over the experimental time frame.

Discussion:

It was found that the HLA-A2 expression on one experimental tumor cell line was more consistently maintained in DMEM than in RPMI. It can be hypothesized that the overall down-regulation in HLA-A2 expression was, in part, due to progressing cell age over the study's allotted timeframe. A significant fluctuation in HLA-A2 expression was also observed. This fluctuation pattern was consistent through all three tested tumor cell lines. The fluctuation in expression may have been a side effect of the simultaneous use of the cells for cytotoxic assays during same time period as the media experiment. The cells were not reused for assays; however, the cells were periodically re-trypsinized and aliquoted for other experimental assays. Cellular stress in such a manner can cause sudden drops in antigen expression (Ahamad et al., 2004).

In analyzing the methodology of this study, one component could address the fluctuation and downregulation of antigen expression that was observed. The protease, trypsin, was used to dissociate adhesive cell monolayers from the bottom of the culturing flasks. If cells are incubated for a short period of time, they may feel minimal effects. Yet, a study performed by Canavan et al., (2005) showed that enzymatic digestion used to lift the monolayer is damaging to both cells and extracellular matrices in bovine aortic endothelial cells. Similarly, Huang et al., (2010) found that the trypsin's proteolytic activity has the potential to cleave cell surface proteins. Taking into consideration that HLA-A2 is a surface antigen, it is possible that the HLA-A2 was continually being damaged due to repeated trypsinization, resulting in the gradual downregulation of the antigen throughout the study. If the HLA-A2 was removed or damaged due to trypsinization prior to the staining and detection of the antigen, the true level of

expression may have been significantly different than was observed. This discussion point is strengthened by the peculiar expression reading on day twenty-eight of the study. After a longer period of recovery from previous trypsinization, the cells may have recovered and/or balanced their HLA-A2 expression to a greater degree. This argument could explain the universally high HLA-A2 expression observed throughout the cell lines tested on day twenty-eight.

The exact reasons behind HLA-A2 downregulation as a function of immune evasion are still relatively unknown and require further study (Möller and Hämmerling, 1992). Therefore, it can only be speculated as to why one medium may consistently maintain HLA-A2 expression to a greater degree. As is suggested by Möller and Hämmerling (1992), the expression of MHC I by tumors is required for an operational T cell response. It is also known that tumors will use HLA hypoexpression to intensify malignancy, as well as evade cytotoxic T cell response (Möller and Hämmerling,1992).

Given that nutritional requirements may impact cellular functions, similarities and differences in media composition must be taken into account when considering their potential impact on antigen expression (Meenakshi, 2013). DMEM is a basal medium, inferring that it contains no inherent growth promoting agents and/or complete proteins. However, it does have a high concentration of amino acids and vitamins. Therefore, when DMEM is supplemented with a serum it becomes a complete medium. DMEM was first used in the culturing of embryonic mouse cells, and is now commonly used for the culture of fibroblasts in tumorigenicity, gene expression, and virus studies (Meenakshi, 2013). DMEM utilizes a bicarbonate buffer system similar to that used by the human body, and maintains the solution at a pH of 7.4 (Meenakshi, 2013). RPMI medium is

used in a wide variety of study areas. It was originally developed for the culture of blood lymphocytes. Now, it is commonly used in cellular differentiation and signaling studies (Meenakshi, 2013).

Both DMEM and RPMI contain phenol red as a pH indicator, allowing for visual monitoring of pH between the passaging of cells (Meenakshi, 2013; Reznikov, 1972). Should the pH drop below the optimum 7.4, the medium becomes more yellow in color. Conversely, should the environment become too basic, the medium will appear to be a deeper purple (Meenakshi, 2013; Reznikov, 1972). Though RPMI uses a sodium bicarbonate buffering system as well, it is adjusted to balance the pH slightly lower than 7.4 (Meenakshi, 2013). This slight difference in pH balance could have affected HLA-A2 expression, and should be investigated in following studies.

Both mediums were supplemented with the essential amino acid, L-glutamine. This amino acid provides nitrogen needed to build nucleotides and NAD derivatives (Lane et al., 1987; Meenakshi, 2013). It can also serve as an alternate source of energy for cellular metabolism (Lane et al., 1987; Meenakshi, 2013). However, L-glutamine is a relatively unstable amino acid which forms ammonia byproduct upon its degradation (Pasieka and Morgan, 1959). A high ammonia levels resulting from L-glutamine overload can be deleterious to cells (Pasieka and Morgan, 1959). If too much L-glutamine were added to one or both of the experimental media batches, HLA-A2 expression could have been impacted.

Both the DMEM and RPMI were also supplemented with antibiotics. Antibiotics are commonly added to cell culture media to prevent unwarranted contaminant and/or bacterial growth (Perlman, 1979). Yet, if the same antibiotics are overused in a lab, the

bacteria and mycoplasma contaminants can become resistant (McGarrity, 1976). As a result, resistant contaminants could be growing in culture without one's knowledge. It has also been discovered that some antibiotics may potentially impede the metabolism of select cells (Masters and Stacey, 2007). Should the tumor lines used for this study have been infected with a resistant mycoplasma or other bacterial strain, antigen expression could have been subsequently affected. Similarly, if the antibiotics used caused adverse side effects, antigen expression could have been affected in this manner as well.

The DMEM used for this study was additionally supplemented with non-essential amino acids (NEAA) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, commonly known as HEPES. Though most cells produce non-essential amino acids themselves, when self-produced NEAA levels become low, cells must consume extra glucose to make up the difference in production (Cyagen Biosciences, 2014). There is therefore an increase in the production of metabolic byproducts. So, the addition of NEAA can minimize the buildup of these metabolic byproducts (Cyagen Biosciences, 2014). The addition of NEAA to DMEM may have given the cells in this medium an advantage, facilitating better antigen expression. HEPES is an additional chemical buffering system that better maintains physiological pH under conditions where environmental carbon dioxide may fluctuate (Shipman, 1969). The incubators housing the cells for this study were being opened and closed frequently, affecting the carbon dioxide balance in the cellular environment. Again, the addition of HEPES to DMEM may have given the cells grown in this medium an advantage over those grown in RPMI.

Though much of this information still demands further investigation, several studies have already demonstrated the effects of culture media on cellular processes,

thereby affecting expression of genes and antigen expression. For DNA metabolic pathways to function properly, specific micronutrients are absolutely necessary (Arigony et al., 2013). Such micronutrient requirements may be better fulfilled by some media rather than others. According to Arigony et al., (2013), supplemental fetal bovine serum is a beneficial additive to provide for micronutrient requirements. Fetal calf serum was added to both media in this study. However, adding more fetal calf serum to DMEM (> 10%) may aid in antigen expression stabilization. Takano et al., (2007) developed a medium specific for the recovery of nephrin expression in murine podocytes. Similar to this current study, Takano et al., (2007) found that DMEM promoted greater activity of nephrin than did RPMI – 1640. Nephrin was activated and the expression of such was maintained for an extended period when podocytes were cultured in a DMEM based media.

Although this study questions the effects of media upon HLA-A2 expression in tumor cells, speculations can still be made about the mechanisms surrounding potential media driven effects on antigen expression. Several ideas should be explored for further. On lymphocytes, the allele-specific expression of HLA at the mRNA and protein levels was investigated in striated muscle satellite cells (Isa A. et al., 2010). Interestingly, it was specified that the regulation of HLA was dictated by post transcriptional control (Isa et al., 2010).

Building upon these ideas, further justification of DMEM's advantageous use can be made. Perhaps DMEM is more apt at nutritionally supporting the mechanisms involving post-transcriptional antigen packaging and transport. The transporter proteins TAP1 and TAP2 are associated with antigen processing in the Golgi apparatus and serve

to shuttle peptides before their combination with the MHC I molecules (Thor Straten et al., 1997). Thor Straten et al., (1997) suggest that the mechanism of MHC I downregulation could be due to the loss of TAP1 and TAP2. Corrias et al., (2001) utilized a neuroblastoma cell line to test the regulation of HLA-class I and beta 2 – immunoglobulin. Several TAP1/2 and tapasin knockout mutants were created and subsequently demonstrated post-translational influences upon antigen expression (Corrias et al., 2001). The study concluded that there may be several complicated mechanisms that play a role in HLA expression maintenance (Corrias et al., 2001). Kageshita et al., (1999) analyzed the LMP2/7 protein subunits (important in melanoma antigen peptide presentation to cytotoxic T cells), in conjunction with the TAP1/2 transporter subunits. It was found that the TAP1/2 is directly associated with melanoma cell fate in relation to cytotoxic T cell escape (Kageshita et al., 1999). The TAP1/2 transport proteins may be post-translationally influenced by characteristic factors of the culture medium used, making antigen transport and packaging more consistent. Further study of these suggested facets is needed to confirm or reject the root of any advantageous characteristic of DMEM and/or its supplements.

The intricacies of HLA-A2 expression need to be addressed when considering adoptive T cell immunotherapy. Though culture media may appear to be only a minute piece in the scheme of immunotherapy research, they could prove to be important components in laboratory methods. Cell culture is vital to this realm of biomedical research, and without healthy cells, the research will progress no further. Culture media cannot be selected at random when preparing for a laboratory experiment. A cell line's individual needs must be considered, and a media must be tailored to fit those needs.

Therefore, it is a worthwhile investment to research the mechanisms and complexities involved in culture media and their effect on antigen expression in experimental cell lines.

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