

**Immunohistochemical characterization of melanomas in bigenic mice containing activated
Cdk4 and keratinocyte specific ablation of Retinoid-X-Receptor alpha (RXRa) protein**

With international consideration of

**Differences in Societal and Behavioral Attitudes toward Skin Cancer between Japanese
American and Japanese**

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Vananh M. Nguyen

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OVERVIEW OF INTRODUCTION

This is a combined thesis for both disciplines of BioResource Research (BRR) Interdisciplinary and International Degree (ID) Programs at Oregon State University (OSU). The first chapter encompasses the BRR research, which is a preliminary study utilizing immunohistochemical method to characterize melanomas in bigenic mice with activated Cdk4 protein and Retinoid-X-Receptor α (RXR α). The second chapter is the ID research, which consists of a survey sent to OSU and Akita International University (AIU) to examine the cultural effects on personal preferences in relation to skin cancer.

CHAPTER 1

Immunohistochemical characterization of melanomas in bigenic mice containing activated Cdk4 and keratinocyte specific ablation of Retinoid-X-Receptor alpha (RXR α) protein

SUMMARY

Vitamin A deficiency in humans and animal models results in epithelial squamous metaplasia prone to malignant conversion (Curtin et al., 2005). Retinoid, an active vitamin A derivative, can function as a chemo-preventive agent and is important in controlling cell growth, proliferation and differentiation. The Retinoid-X-receptor (RXR) is a central coordinator in mediating the cellular activity of retinoids *in vivo*. It has been previously shown that the loss of RXR α in epidermal keratinocytes leads to the development of melanocytic nevi. This study investigated the synergistic effect of RXR α ablation and activated Cdk4 in both development and progression of cutaneous melanoma. In combination with activated Cdk4, the melanocytic tumors increase in size and have greater malignant potential.

INTRODUCTION

Melanoma, the deadliest form of skin cancer, is the 5th most common invasive cancer diagnosed in Oregon. In 2006, Oregon is ranked 7th in incident rate nationally. The Oregon melanoma mortality rate was 26% higher than was the national rate (Cancer in Oregon, 2006).

Skin is the largest organ in the body and forms a barrier between the organism and the external environment. One important cell type within the skin is the melanocyte located within the basal

layer of the epidermis, as well as in the hair follicles. Melanocytes manufacture melanin that helps to determine both hair and skin pigmentation. One melanocyte supplies approximately 36 keratinocytes with melanin granules, and this transferring process between melanocytes and keratinocytes also aids in the prevention of UV-induced genetic mutations (Scott, 2006). Previous study indicated that there is a paracrine signaling in this transferring process that is crucial in melanoma development.

In order to investigate the transferring process between melanocytes and keratinocytes in relation to melanoma Vitamin A deficiency in humans and animal models results in epithelial squamous metaplasia prone to malignant conversion. Retinoid, an active vitamin A derivative, can function as a chemo preventive agent and is important in controlling cell growth, proliferation and differentiation (Curtin et al. 2005).

The steroid hormone receptor protein Retinoid-X-receptor α (RXR α) is a central coordinator in mediating the cellular activity of retinoids *in vivo*. RXR α is known to heterodimerize with other nuclear receptor (NR) superfamily members (Champon, 1996). NRs are transcription factors that have diverse roles in regulation of cell growth, development and homeostasis. The heterodimerization between RXR and NRs occupy corresponding response elements present in the promoters of target genes, thus activating or repressing gene transcription (Mangelsdorf et al., 1995; Altucci and Grenemeyer, 2001). This transcriptional regulation plays a vital role in the cellular signaling controlling skin carcinogenesis. The RXR α -null mutation (RXR $\alpha^{-/-}$) is embryonic lethal in mice, thereby preventing analysis of *in vivo* function of this protein in skin homeostasis and diseases (Sucov et al., 1994).

Cyclin-dependent kinases (CDKs) belong to a group of protein kinases that are involved in cell cycle control and have been considered a potential target for anti-cancer medication (Loyer et al., 2005). Cyclin-CDK inhibitors (CKIs), such as p16INK4a, are involved in the negative regulation of CDK activities, thus providing a pathway through which the cell cycle is negatively regulated (GenomeNet, 2010). Cyclin-dependent kinase 4 (CDK4), a product of the Ink4a locus, is involved in G1 phase of the cell cycle. CDK4 has been proven to play a vital role in certain types of cutaneous melanoma (Kamb et al., 1994) through a point mutation of arginine (R) to cystine (C) ($\text{Cdk4}^{\text{R24C/R24C}}$) that inhibits G1/S regulator p16, resulting in an increase in cell cycle activity (Wolfel, Hauer et al. 1995; Zuo, Weger et al. 1996). In this project, we investigated the contributions of $\text{Cdk4}^{\text{R24C/R24C}}$ and keratinocytic RXR α to influence metastatic progression in a mouse model.

$\text{RXR}\alpha^{\text{ep-/-}}$ mice, with an epidermal specific deletion of the RXR α gene, were generated by using Cre-Lox technology (Metzger et al, 2003). $\text{Cdk4}^{\text{R24C/R24C}}$ mice were bred to $\text{RXR}\alpha^{\text{ep-/-}}$ to produce $\text{RXR}\alpha^{\text{ep-/-}}/\text{Cdk4}^{\text{R24C/R24C}}$ bigenic mice. A two-step chemical tumorigenesis protocol was used to study initiation, promotion, and progression of mouse skin tumors. The initiation stage is achieved through a single topical application of tumor initiator 7, 12-dimethyl-benz[a]antracene (DMBA). Promotion is accomplished by repeated topical application of the tumor promoter 12-0-tetradecanoylphorbol-13 acetate (TPA). It causes a sustained hyperplasia and inflammation followed by a selective clonal expansion leading to benign papillomas. The third stage, progression, is the malignant conversion of benign papillomas to invasive squamous cell carcinoma (Indra et al., 2007).

Our hypothesis is that the increased melanocytes proliferation due to ablation of RXR α (Indra et al., 2007) could be due to deregulated cell cycle control, specifically the kinase activity of activated Cdk4. This research thesis focused on the immunohistochemical characterization of melanocytic growths of RXR $\alpha^{ep-/-}$ /Cdk4^{R24C/R24C} mice to evaluate the cooperative effects of aberrant keratinocytic signaling and activated Cdk4 in melanoma metastasis.

MATERIALS AND METHODS

Animal protocol was approved by Oregon State University Institutional Animal Care and Use Committee (IACUC).

Preparation of transgenic mice

The RXR α gene was ablated in the basal layer of the epidermis mice using a keratinocyte-specific promoter using Cre/loxP technology (Metzger et al., 2003) as described in previous study (Indra et al., 2007). Cdk4^{R24C/R24C} was a knock-in mice gene line harboring the activated form of Cdk4 kinase. The knock-out RXR $\alpha^{ep-/-}$ and knock-in Cdk4^{R24C/R24C} mice were put in the same cage to initiate breeding. The progeny RXR $\alpha^{ep-/-}$ /Cdk4^{R24C/R24C} bigenic mice were generated. The RXR $\alpha^{L2/L2}$ mice were used as controls, as well as mice with single mutations (RXR $\alpha^{ep-/-}$ and Cdk4^{R24C/R24C}). There are 8-10 mice in each cohort. All mice were housed in approved University Animal Facility with 12-h light cycles, food and water provided in libitum.

Determination of desired genotypes in mice

Genotyping technique was done as previously described (Li et al., 2000; Indra et al., 2007) with the modification of using tail sample of 10-day old mice instead of dorsal skin.

Next, isolated DNA was amplified using semi-quantitative polymerase chain reaction (PCR) for 26 cycles as previously described (Li et al., 2001).

Two-step chemical tumorigenesis

All mice were shaved and subjected to the two-step chemical tumorigenesis protocol consisting of 50 μ g DMBA in 100 μ l of acetone followed by 5 μ g TPA in 200 μ l acetone 5 days later. The TPA treatment was applied topically twice a week for up to 25 weeks. Mice were sacrificed earlier if they developed melanocytic tumors too rapidly and/or the melanoma was too invasive.

Gross morphology analysis

All mice were shaved weekly with observable number and size of melanocytic tumors recorded (Figure 1 and 2). In order to further characterize the progression of MTs, the radical growth phase (RGP) of MTs was used to detect the growth rate in which tumor cells spread laterally into the epidermis, in addition to the vertical growth phase (VGP) that helped determine the spread from epidermis into dermis. Statistical significance was calculated by an unpaired Student's t-test using GraphPad Prism software.

Collecting samples

Mice were placed in chamber with high isoflurane gas, a anaesthetic gas. Once unconscious, mice were subjected to carbon monoxide gas to induce death. Necropsy was performed on Cdk4^{R24C/R24C} and RXR α ^{ep-/-}/Cdk4^{R24C/R24C} mice groups only. Samples of dorsal skin as well as melanocytic growths were obtained. The tissues were fixed in 4% formaldehyde in PBS (pH 7.2) for 3 hours then dehydrated. Tissues were processed and embedded in paraffin then cut into 5 μ m thick sections using a Microtome.

Histological analysis

Histological analysis was performed using hemoxylin and eosin (H&E) staining to visualize the epidermal thickening and melanocytic growths from both genotypes.

Hydration step was achieved by subjected paraffin sections in histosol, graded EtOH and finally rinsed with dH₂O (Table 1).

	Number of slides immersion	Duration
Histosol	2 times	5 minutes
100% EtOH	2 times	2 minutes
80% EtOH	1 time	2 minutes
50% EtOH	1 time	2 minutes
dH ₂ O	1 time	2 minutes

Table 1. Hydration step of H&E staining

5 µm sections were then stained with Hematoxylene for one minute, washed with dH₂O for two minutes then clarifer for another two minutes. Dehydration step (Table 2) was followed with blueing reagent for two minutes and Eosin (Eosin: EtOH = 50%:50%) for 20 seconds after subjecting slides in water and 95% EtOH, respectively then mount on slides.

	Number of slides immersion	Duration
dH ₂ O	1 time	2 minutes
95% EtOH	1 time	2 minutes
100% EtOH	2 times	2 minutes
Xylene	2 times	2 minutes

Table 2. Dehydration step used in H&E and Immunohistochemistry procedure

All microscopic studies were performed using a Leica DME light microscope and analyzed using the Leica Application Suite software, version 3.3.1.

Immunohistochemical analysis

Formalin-fixed paraffin-embedded (FFPE) protocol was followed. Slides of stained tissue samples were baked for one hour at 60°C. A series of rehydration steps that included immersing slides in histosol then followed with graded concentration of EtOH and finally deionized water (Table 3).

	Number of slides immersion	Duration
Xylene	3 times	5 minutes
100% EtOH	2 times	3 minutes
95% EtOH	1 time	3 minutes
70% EtOH	1 time	3 minutes
dH ₂ O	1 time	3 times

Table 3. Rehydration step of FFPE protocol

Antigen retrieval procedure was done by subjected slides to high temperature (+95°C) water bath for 20 minutes using citrate buffer pH 6.0. Slides were placed in 0.3% H₂O₂ to quench any endogenous activity. In order to block nonspecific antibody binding, slides were washed with 10% normal goat serum PBS-Tween (0.05%) for 30 minutes in humidified chamber at room temperature (RT) then incubated overnight (O/N) at +4°C with rabbit anti-RXRa polyclonal antibody (Santa Cruz, 1/300 dilution). Next 5µm sections were once again washed with PBS-Tween (0.05%) solution 3 times, 5 minutes each before subjected to secondary antibody staining with biotin-labeled goat anti-rabbit

antibody (Jackson ImmunoResearch, 1/1500 dilution) for 2 hours at RT, followed by incubation with a streptavidin-biotin horseradish peroxidase complex (Vector Laboratories). Slides were counterstained with Harris hematoxylin followed by dehydration (Table 2) and mounting.

Paraffin sections were rehydrated and processed as above for immunofluorescence staining studies. Antigen retrieval was performed similarly as above. Difference is that after the hot water bath incubation, slides were bleached at 60°C with 10% H₂O₂ for 30-60 minutes. The rest of protocol was similar to above procedure with primary antibody incubation was followed by three washes with PBS-Tween (0.05%) before addition of the secondary antibodies. Nuclei were counterstained with DAPI. Slides were also rinsed with PBS-Tween (0.05%) and went through dehydration step (Table 2). Slides were mounted with DPX mounting media and allowed to dry O/N. Primary antibodies used for melanocytic proliferation by co-labeling directed antibodies against proliferation marker (Pcna) and the melanocyte-specific enzyme tyrosinase-related protein 1 (Trp1) (Jimenez et al., 1988; Waseem and Lane, 1990). IHC staining was also performed using a cocktail directed against melanoma antigens MART-1 and HMB45 to determine the maglinant nature of the tumors from bigenic mice (Yamazakin et al., 2005). In addition, the endothelial cell-specific-marker CD31 was also used in IHC staining to detect the vascularization levels within tumors (Wang et al., 2008). The secondary antibodies used were goat anti-rabbit CY2 and goat anti-mouse CY3 (Jackson ImmunoResearch). All images were captured at 40X. Sections stained without primary antibody was used as a negative control.

RESULTS

Ablation of keratinocytic RXR α in combination with activated Cdk4 increases melanocytic tumors size

After utilizing the two-step carcinogenesis DMBA/TPA protocol, all mice developed melanocytic tumors (MTs) over the course of study. However, larger MTs were demonstrated on RXR α -ablated mice cohorts ($\text{RXR}\alpha^{\text{ep}^-/-}$ and $\text{RXR}\alpha^{\text{ep}^-/-}/\text{Cdk4}^{\text{R}24\text{C/R}24\text{C}}$) compared to the wild-type control, $\text{RXR}\alpha^{\text{L}2/\text{L}2}$ and the single mutated $\text{Cdk4}^{\text{R}24\text{C/R}24\text{C}}$ mice (Figure 1).

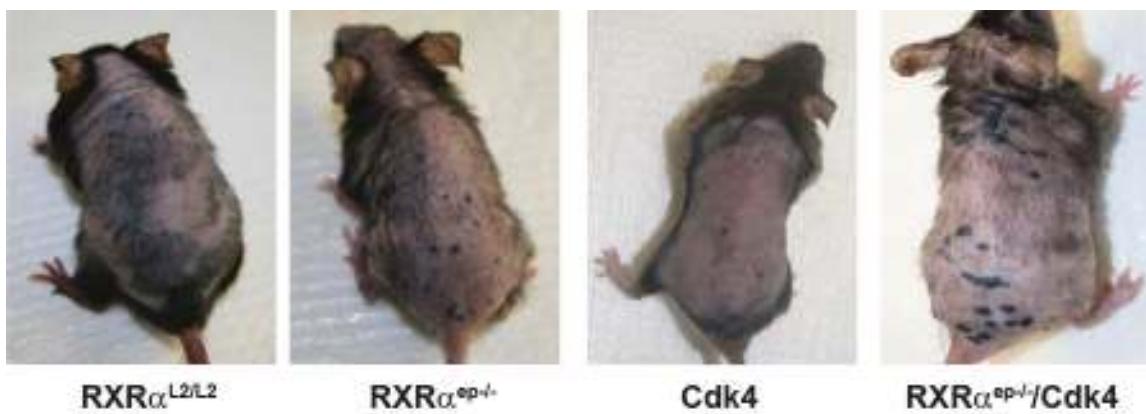


Figure 1. Representative gross morphology of $\text{RXR}\alpha^{\text{epL}2/\text{L}2}$, $\text{RXR}\alpha^{\text{ep}^-/-}$, $\text{Cdk4}^{\text{R}24\text{C/R}24\text{C}}$ and $\text{RXR}\alpha^{\text{ep}^-/-}/\text{Cdk4}^{\text{R}24\text{C/R}24\text{C}}$ dorsal skin after DMBA/TPA treatment.

In addition, a significant greater number of MTs with size larger than 2mm in diameter found in the bigenic mice and $\text{RXR}\alpha^{\text{ep}^-/-}$, comparing to the other two control groups (p-value < 0.05). Furthermore, the bigenic mice also developed a higher number of MTs over 4mm, compared to the $\text{RXR}\alpha^{\text{ep}^-/-}$ mice with p-value < 0.01 (Figure 2).

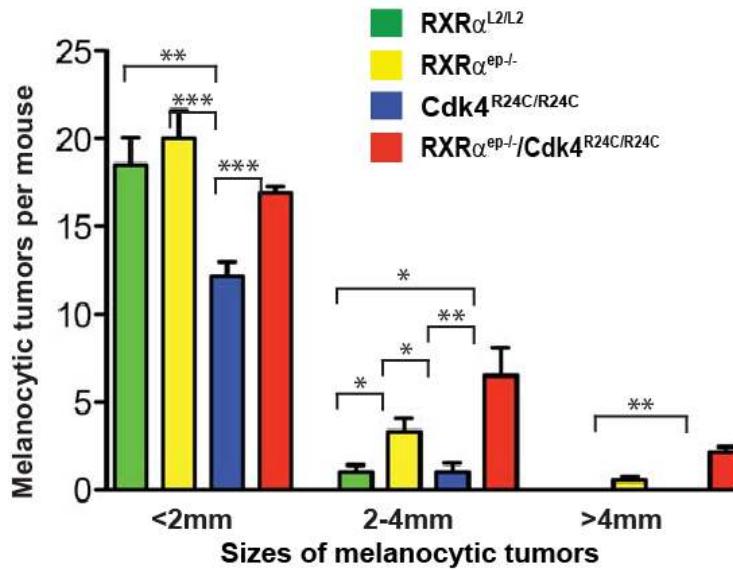


Figure 2. Size distribution of melanocytic tumors in $RXR\alpha^{L2/L2}$, $RXR\alpha^{ep/-}$, $Cdk4^{R24C/R24C}$ and $RXR\alpha^{ep/-}/Cdk4^{R24C/R24C}$ mice after DMBA/TPA treatment. Values are expressed as mean +/- SEM. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Histological and Immunohistochemical analyses of melanocytic tumors from $RXR\alpha^{ep/-}/Cdk4^{R24C/R24C}$ bigenic mice

The H&E staining of MTs from both $RXR\alpha^{ep/-}/Cdk4^{R24C/R24C}$ and $Cdk4^{R24C/R24C}$ genotypes showed the different characterizations of the MTs in dorsal skin (Figure 3). The MTs (black color) were manifested drastically in the dermal layer of the bigenic mice, comparing to the $Cdk4^{R24C/R24C}$ mice. A significant increase (p -value < 0.01) in epidermal thickness from the bigenic mice compared to the mutated $Cdk4^{R24C/R24C}$ alone was also observed (Figure 4). In addition, both the RGP and VGP of the MTs were strikingly higher from the skin of bigenic mice in comparison to the $Cdk4^{R24C/R24C}$ mice alone (Figure 5) (p -value < 0.05 and p -value < 0.01 respectively).

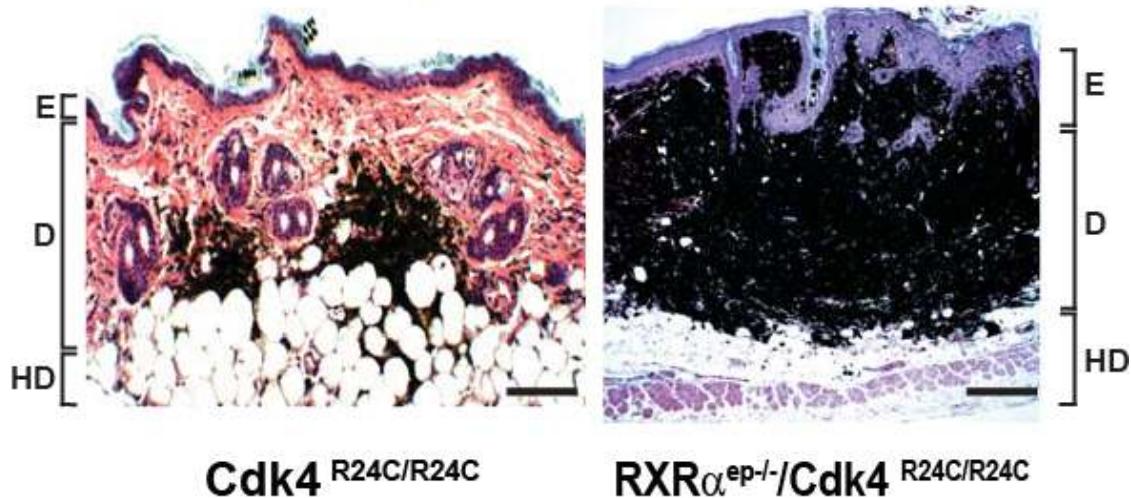


Figure 3. Histological analyses of melanocytic tumors from $Cdk4^{R24C/R24C}$ and $RXR\alpha^{ep-/-}/Cdk4^{R24C/R24C}$ mice.

E, epidermis; D, dermis; HD, hypodermis. Scale bar = 62 μ m.

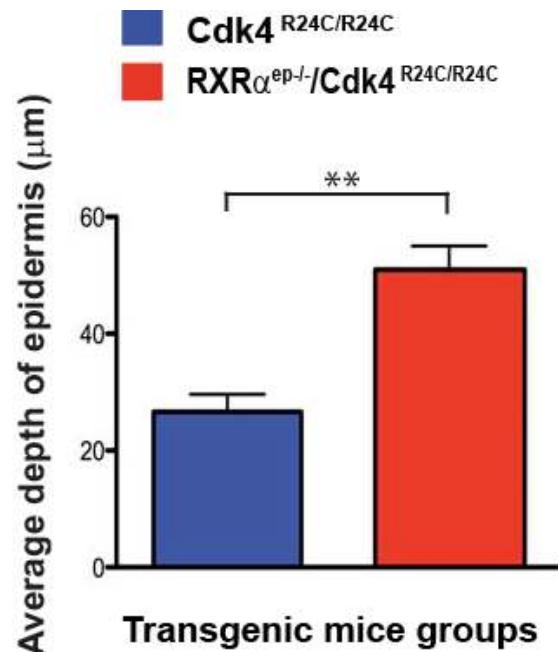


Figure 4. Average depth of epidermis comparison between $Cdk4^{R24C/R24C}$ and $RXR\alpha^{ep-/-}/Cdk4^{R24C/R24C}$ mice.

** = $p < 0.01$

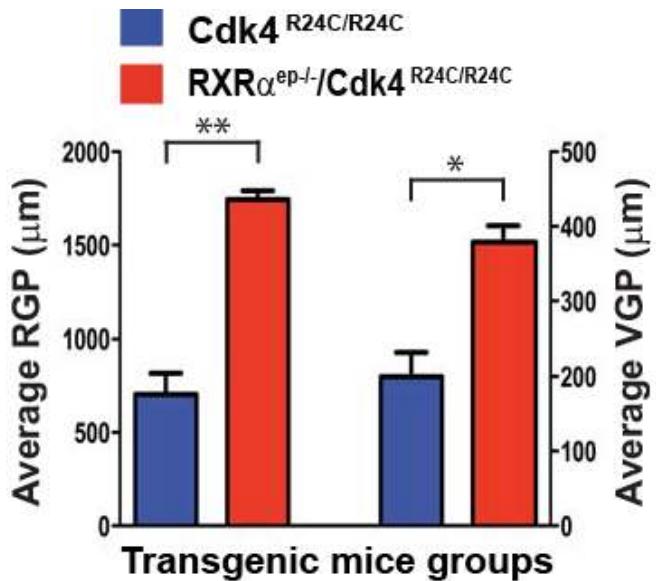


Figure 5. Radical growth phase (RGP) and vertical growth phase (VGP) of melanocytic tumors in $\text{RXR}\alpha^{ep-/-}/\text{Cdk4}^{\text{R}24\text{C}/\text{R}24\text{C}}$ mice. * = $p < 0.05$, ** = $p < 0.01$

The melanocytic proliferation was characterized using IHC technique by co-labeling the paraffin sections with PcnA and Trp1. The results showed a significantly higher percentage of co-labeling between PcnA and Trp1 in $\text{RXR}\alpha^{ep-/-}/\text{Cdk4}^{\text{R}24\text{C}/\text{R}24\text{C}}$ skin than in $\text{Cdk4}^{\text{R}24\text{C}/\text{R}24\text{C}}$ alone with a P-value < 0.01 (Figure 6). Similarly, the IHC staining for maglinant melanocytes using antibody directed against melanoma antigens MART-1 and HMB45 also showed a much higher staining in all of the MTs in the bigenic mice $\text{RXR}\alpha^{ep-/-}/\text{Cdk4}^{\text{R}24\text{C}/\text{R}24\text{C}}$ than the $\text{Cdk4}^{\text{R}24\text{C}/\text{R}24\text{C}}$ mice, indicating a larger number of maglinant cells in these tumors (Figure 7). Also, increased vascularization was detected by anti-CD31 antibody in melanocytic tumors from bigenic mice compared to $\text{Cdk4}^{\text{R}24\text{C}/\text{R}24\text{C}}$ alone (Figure 8). The above results suggest that MTs from those with $\text{RXR}\alpha$ ablated in cooperation of activated Cdk4 ($\text{RXR}\alpha^{ep-/-}/\text{Cdk4}^{\text{R}24\text{C}/\text{R}24\text{C}}$ genotype) have a higher maglinant potential compared to those from the individual mutated groups ($\text{RXR}\alpha^{ep-/-}$ and $\text{Cdk4}^{\text{R}24\text{C}/\text{R}24\text{C}}$).

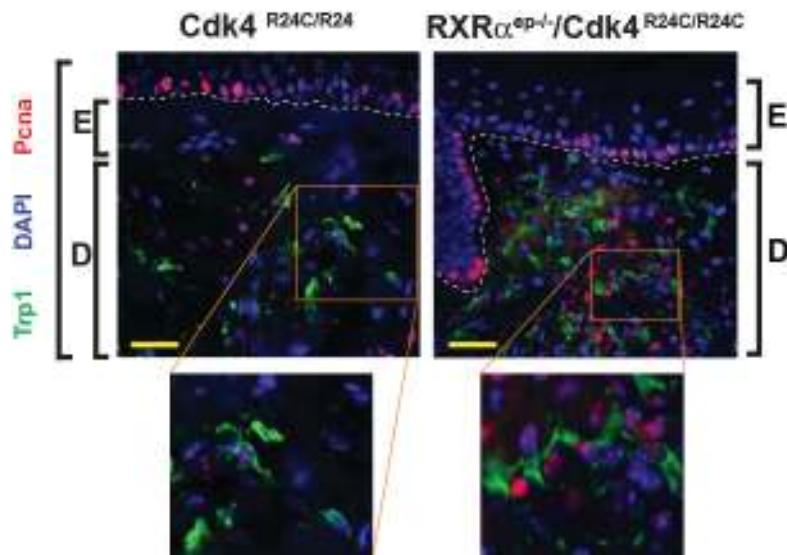


Figure 6. Enhanced melanocyte proliferation in melanocytic tumors from $\text{RXR}\alpha^{\text{ep}^{-/-}}/\text{Cdk4}^{\text{R24C/R24C}}$ mice as determined by co-labeling with anti-Trp1 [green] and anti-Pcpna [red] antibodies. Scale bar (in yellow) = 33 μm . White dashed lines, artificially added, indicate epidermal-dermal junction. Blue color corresponds to DAPI staining of the nuclei.

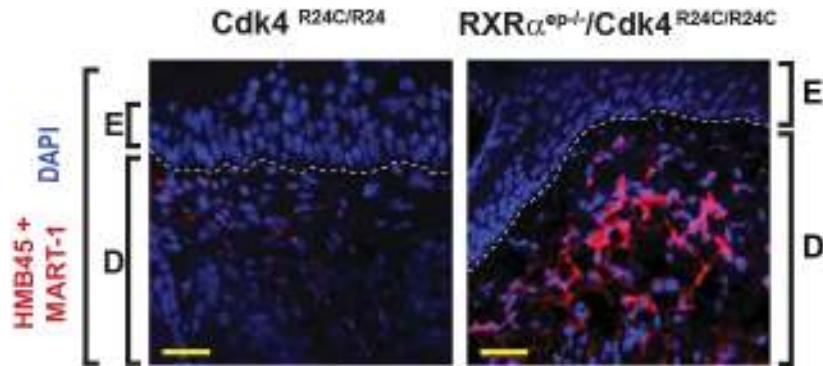


Figure 7. IHC staining for malignant melanocytes using a cocktail of antibodies directed against melanoma antigens HMB45 and MART-1 [red]. E, epidermis; D, dermis.

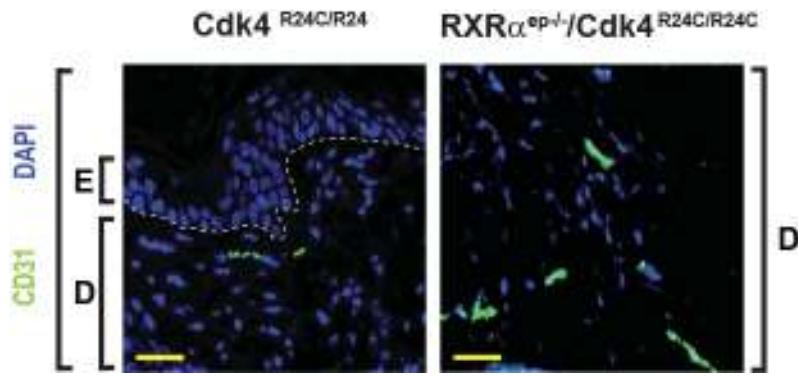


Figure 8. Increased vasculization in melanocytic tumors from $\text{RXR}\alpha^{\text{ep-/-}}/\text{Cdk4}^{\text{R24C/R24C}}$ mice was detected using anti-CD31 antibody (green) compared to $\text{Cdk4}^{\text{R24C/R24C}}$ mice. E, epidermis; D, dermis.

DISCUSSIONS

Formation of metastatic melanoma in $\text{RXR}\alpha^{\text{ep-/-}}/\text{Cdk4}^{\text{R24C/R24C}}$ mice

The deletion of $\text{RXR}\alpha$ in keratinocytes contributes to the development of cutaneous melanoma after carcinogen treatment (Indra, Castaneda et al. 2007). Since melanocytes in the previous study were not genetically modified, it suggests an influence of paracrine signal(s) from $\text{RXR}\alpha$ -ablated epidermal keratinocytes on melanocyte biology during melanomagenesis.

Our present study demonstrated that the addition of activated Cdk4 contributes to the malignant transformation and metastatic potential of proximal melanocytes. IHC studies on human tissues confirmed decreased expression of nuclear receptor $\text{RXR}\alpha$ in melanocytes during melanoma progression, thereby corroborating the results reported earlier (Chakravarti et al., 2007). In addition, we showed downregulation of $\text{RXR}\alpha$ expression in tumor adjacent keratinocytes from human *in situ* and malignant melanoma samples. Our results suggest a protective role of keratinocytic $\text{RXR}\alpha$ against aggressive

tumor formation, the lack of which resulted in a significantly increased RGP and VGP in the bigenic RXR α ^{ep-/-}/Cdk4^{R24C/R24C} tumors. In order to achieve vertical growth, the nevus must acquire characteristics of both tumorigenicity and mitogenicity, properties that enable cellular proliferation within a foreign matrix (Hearing and Leong 2006). The increase in Pcn a^+ /Trp1 $^+$ cells within the RXR α ^{ep-/-}/Cdk4^{R24C/R24C} melanocytic tumors verified the presence of a large population of proliferating melanocytes. Increased staining against melanoma antibody cocktail HMB45 and MART-1 detected in the RXR α ^{ep-/-}/Cdk4^{R24C/R24C} tumors correlated well with the higher VGP in those mice. Similarly, CD31 staining in the bigenic skin implied that the larger MTs formed in those mice would necessitate additional vascular formation for nutritional support. Altogether, these data confirm that melanocytic tumors formed in the absence of keratinocytic RXR α , and in the presence of activated Cdk4, have increased metastatic capabilities compared to the control mice. It has been previously shown that the loss of the transcriptional regulator Taf4 in epidermal keratinocytes can lead to melanocytic tumors with invasion of lymph nodes after carcinogen treatment (Fadloun, Kobi et al. 2007). It remains to be determined whether Taf4 and RXR α act through the same or different paracrine/juxtacrine pathways to regulate melanocyte homeostasis. Activating mutations in N-Ras and its downstream effector B-Raf are frequent events in human nevi and melanomas (Papp et al., 1999; Demunter et al., 2001; Garnett and Marais, 2004). Importantly, N-Ras and B-Raf mutations are mutually exclusive, strongly suggesting that both oncogenic activities are in the same linear pathway deregulating the mitogen activated protein kinase (MAPK) pathway. Mice bearing oncogenic N-Ras^{Q16K} or a B-Raf^{V600E} have been shown to develop melanomas mimicking the genetics and pathology

of the human disease (Ackermann et al., 2005; Dhomen et al., 2009). It is possible that keratinocytic RXR α mediated NR pathway(s) also cooperate with the N-Ras^{Q16K} or B-Raf^{V600E} driven MAPK pathway towards melanoma progression in humans.

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CHAPTER 2

Differences in Societal and Behavioral Attitudes toward Skin Cancer between Japanese American and Japanese

APPROVED:

Dr. Nancy Rosenberger, Mentor

Date

David Bernell, International Degree Director

Date

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Vananh M. Nguyen

Date

AN ABSTRACT OF THE THESIS OF

Vananh Nguyen for the degree of Bachelor of Arts in International Studies in Bioresource
Research presented on June 6, 2011.

Title: Differences in Societal and Behavioral Attitudes toward Skin Cancer between Japanese
American and Japanese

Abstract approved: _____
Dr. Nancy Rosenberger

The rate of melanoma among Asian American has been steadily increasing in the past five years, while in other Asian countries, such as Japan, the incident rate remains relatively the same. Does the culture and societal attitudes toward skin cancer make a difference in the incident rate of melanoma? This survey research investigated this disparity. The populations of interest were Japanese American and Japanese living in Japan. Skin tone preference, the usage of SPF-contained products, hat and umbrella, skin whitening and tanning products, opinion on white and tanned skin and perceptions on skin cancer were used as premises for comparison. Contrary to the neutral opinions from Japanese American, the majority of Japanese view fondly of white skin over tanned skin. There were some distinct differences in the practice of using umbrella, whitening and tanning skin products. However, there was not a significant difference in the use of SPF-contained products. Additionally, both populations also share similar perceptions about skin cancer.

Beauty value generates societal and behavioral attitudes. Since white skin preference is an important aspect valued not just as an esthetic matter but also as an indicator of social class, skin care practice is prioritized in beauty care in Japan. As a result, certain behaviors in skin

cancer prevention practices found in native Japanese diverge from Japanese living in other Western countries such as America. The results for this project contribute to the understanding of cultural effects on skin protection and prevention. It has a potentially important role in public health promotion and prevention in America.

RATIONALE AND PERSONAL INTERESTS

As a student in dual-degree Bioresource Research and International Studies, I am both interested in science, specifically in medicine as well as international related issues.

Upon living and working in both countries, I am aware of the differences in health care system as well as attitude toward skin cancer prevention between American and Japanese.

Asian people, especially women, prefer lighter skin tone as a sign of beauty. Americans in general view slightly tanned skin is a sign of well-being. While not all Asian American prefer darker skin tone, some do seek tanning, naturally or artificially, to have a healthier-looking skin color. In Japan, as well as other East Asia countries, the obsession of whiter skin is shown in media and in the abundance of whitening skin products available in market.

Given the high precipitation on average per year for both Akita prefecture and Oregon, umbrella is the most common protection during the wet seasons. However, umbrella in Japan has another use: against the sun. During my stay in Japan in summer 2009, I occasionally spotted several women who wore long sleeves and gloves in addition to the umbrella on their hands to shield them from the blazing sun of summer.

Are these differences the sources of the lower incident rate in melanoma in Japan? What factors contribute to the higher risk development of melanoma? They are my questions of interest that lead to the conduction of this research.

INTRODUCTION

Melanoma is most rapidly increasing of all cancers in America¹. While there are differences in skin characterizations of different ethnics that greatly contribute to skin cancer risks, the rate of Asian American who develops melanoma has been slowly increased in the past five years⁴. However, at other Asia countries, especially in Japan, the rate has remained relatively the same with insignificant rise in female (Figure 1). Do cultures have an effect in this disparity? Is there a distinct difference between the two countries?

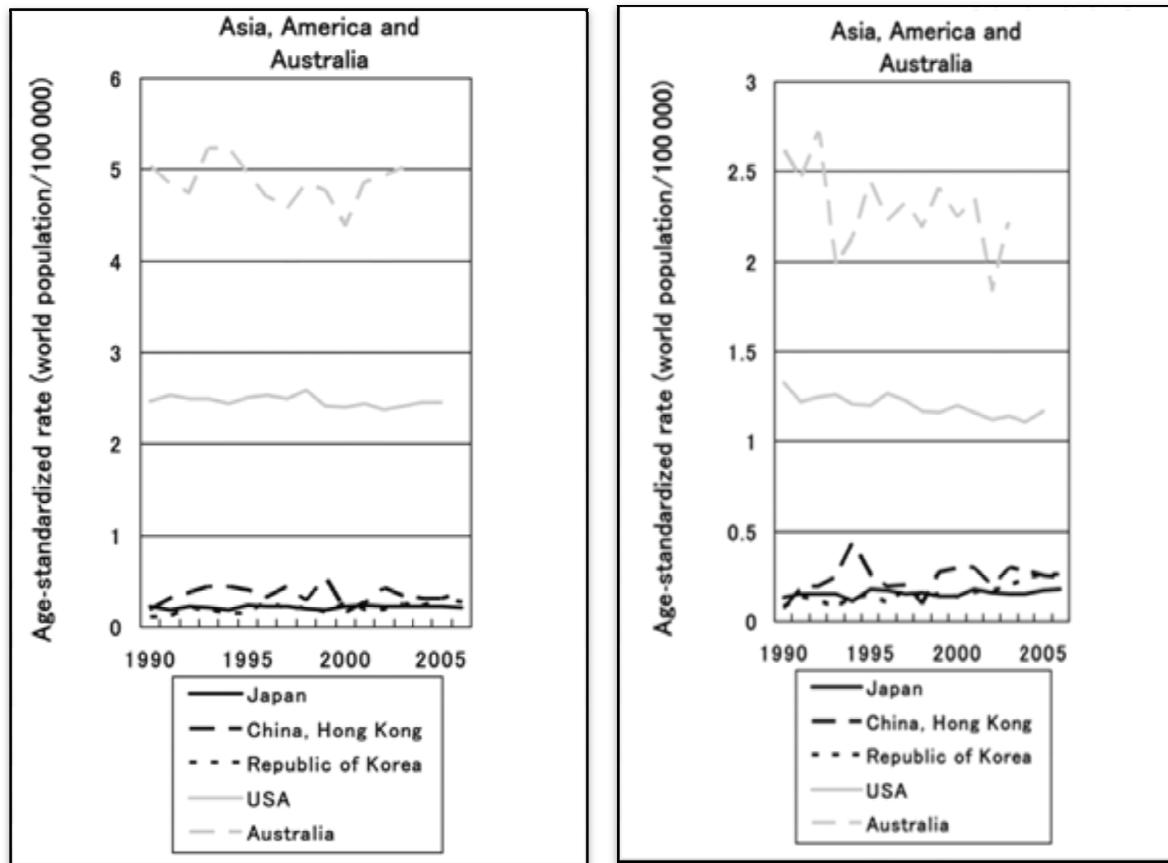


Figure 1: Trends in age-standardized melanoma of skin cancer mortality rates over time in different countries. Male (left), Female (right)

By performing appropriate statistical tests, with a sample size of Japanese American students at Oregon State University, the desired population of Oregon residents will be able to be evaluated. Similar tests will be performed for the targeted population in Akita prefecture, Japan. The sample is Japanese students at Akita International University.

Akita prefecture (Akita-ken) is located in the Tohoku region of northern Japan. Although different in area size and population, there are many similarities between Akita-ken and Oregon. Their climates are quite similar, especially the high temperature during the hottest months of the year, from June to September, as indicated in tables below. The resemblance in climates is the important factor in comparing the behaviors toward skin protection of the population.

This research compares and contrasts the cultural and social differences in America and Japan. This project has a potential to play an important role in public health promotion. The research will contribute to better understanding of cultural effects in skin cancer that might be an important factor in promoting health care and prevention of this deadly disease.

Statistic	Units	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Temperature Mean Value	F	31.3°	31.5°	37°	48.4°	57.6°	65.5°	72.7°	75.9°	67.3°	55.6°	45.3°	36.5°
High Temperature Mean Value	F	36.3°	36.9°	43.9°	56.5°	65.7°	72.9°	79.3°	83.5°	75.2°	64.4°	52.7°	42.1°
Low Temperature Mean Value	F	26.2°	26.1°	30.4°	40.1°	49.3°	58.6°	66.6°	69.3°	60.1°	47.7°	38.5°	31.1°
Precipitation Mean Monthly Value	Inches	5.3	3.8	4	5.5	4.7	4.8	7.7	7.8	7.5	6.1	7.4	7.1

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Table 1: Average weather data of Akita Prefecture, Japan

Statistic	Units	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Temperature Mean Value	F	39.4°	42.8°	46°	49.3°	54.7°	61°	65.7°	66.2°	61.5°	53.1°	45.1°	39.7°
High Temperature Mean Value	F	45.5°	50.4°	54.9°	59.5°	66°	73°	80.2°	81.1°	75.4°	64.2°	52.2°	45.7°
Low Temperature Mean Value	F	33.1	35.1	37	39.2	43.2	48.6	51.1	51.3	47.8	41.7	37.9	34
Precipitation Mean Monthly Value	Inches	7.1	5.2	4.7	2.7	2	1.3	0.5	0.9	1.6	3.2	7.1	8

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Table 2: Average weather data of Corvallis, Oregon, USA

BACKGROUND

Epidemiological study

While Asian/Pacific Islanders who live in the United States of America are among the lowest group in skin cancer incident, they have the highest annual percentage change (6%)², comparing to other ethnic minorities. Fair skin was revealed as a risk factor for the development of non-melanoma skin cancer in Japanese population^{1,2}. However, according to an incident report done on non-melanoma skin cancer in Hawaii in 1995, the incidence for skin cancer was nearly 45 times more for the Japanese Hawaii residents than that of the native Japanese population. This research revealed the importance of high UV radiation exposure to the risk of getting non-melanoma skin cancer, as proof of darker skin color in Japanese Hawaiian than the native Japanese population.



Figure 2: The use of parasols or umbrella seen in Japanese men. Courtesy of japan-trends.com²

have another use: against the sun. It is not uncommon to see people walking on the street, men and especially women, with an umbrella on their hand to shield them from the blazing sun of summer. Some of the Singaporean

students in the same program also had the same practice, while other students from the West, myself included, were surprised (and slightly amused) by the scenes.

Interestingly, a long time ago, traditional Japanese parasol that made of paper was used to fight off the brutal heat of summer. Raincoat that made of straw was used in rainy season instead. The beauty standards of Japanese people that prefer white skin over dark skin perhaps impinge upon this behavior.

The Use of Umbrella

Given the high precipitation on average per year for both Akita prefecture and Oregon, umbrella is the most common protection during

the wet seasons in both areas.

However, umbrellas in Japan



Figure 3: Singaporean students in 2009 AIU Summer program shield themselves from the sun with umbrella. Copyright @ 2009 Nguyen



What is Beauty?

Japanese people, especially women, prefer fair skin tone as a sign of beauty. Take *geisha* for example. *Geisha* are elegant, high-class entertainers in Japan. The most recognizable characteristics for *geisha* are their white make-up, elaborate kimono and hairstyle. The white make-up used by both *geisha* and *maiko*(*geisha* apprentice) shows the most traditional Japanese femininity.

Figure 4: A *geisha*. Courtesy of Frantisek Staud⁷.

Geisha is hailed as the classic ‘ultimate beauty’ symbol in Japan. The *bihaku* or “beautiful white” trend is to emulate this porcelain-pale complexion of *geisha*. An old Japanese proverb states that “white skin makes up for seven defects”⁶. Throughout history, a woman’s social status in Japan has been measured by the clarity of her complexion. The Japanese obsession with ultra-white skin stems from traditional aesthetics. Whiter skin appearance in women is often waxed over in writing and music. Many believe that Asian women pursue white skin to emulate the stereotypically Caucasian beauty. Looking “Westerner” has widely believed as the key to success in the East. Others believe in a social class difference that lighter complexion allude. Wealth, higher education levels and refinement are associated with lighter complexion while darker skin suggests an outdoor labor life. In the past, if Japanese women wanted to be seen as a member of high society, she would paint her face white to create the illusion of unsullied skin. Most recent theory however, proposes the innocence and femininity of fair-skinned emit an image of youthfulness and attractiveness to the opposite sex that seem to be consistent throughout Japanese history.

Interestingly, a fashion style that started in the early 1990s and peaked in early 2000s called *ganguro* ‘blackface’ shook the Japanese concepts of beauty⁶. Girls were seen with blacken face with heavy, shimmering makeup with blond or white hair. Figure 4 is an example of a teenager on the street of Shibuya. Some researchers in the field of Japanese social and/or studies believe that *ganguro* as a fashion style is the younger generation’s

revenge against traditional Japanese society; others believe that it is just some Japanese girls’ imitation of



Figure 5: A *ganguro* teenager seen on the street in Tokyo. Courtesy of Kevin from Music and Culture blogspot.⁶

some elements of an African woman’s appearance to be a ‘woman’, and still others believe that it makes girls *kawaii* (cute) or cool because it makes them look different from others. Although researchers may view *ganguro* from different perspectives and offer various explanations, *ganguro* has been understood as an explicit expression of self-identity instigated by some Japanese girls’ resentment at being neglected, ignored, isolated, and rule-governed or constrained. Although there is no definite timeline for this fad but it was short-lived and Japanese women quickly came back to the *bihaku* trend until today.

The general attitude of American, including people of Asian descendants, is the natural to tanned skin color reveal one’s well-being. While not all Asian American prefer darker skin tone, many do sunbathe or seek tanning to have a healthier-looking skin color. These UV-seeking activities undoubtedly play an important role in the higher risk of getting skin cancer, thus, contributes to the increase incident rate of these deadly diseases.

Skin whitening and tanning

The obsession of whiter skin in general Japanese population (as well as in other Asia countries) is observed in the regular use of high SPF-contained products, even in winter and the wide-range of whitening skin products on market. Japanese is the leader in skin whitening

products in the world. They are generally cheaper than the ones in America and are available at most pharmacies, supermarkets and some even in 24/7 convenient stores. In contrast, in America, it is much easier to find tanning products in a store than skin whitening products.



Figure 6: The “Skin Milk” products that contained high SPF at a convenient store. Copyright 2009 @ Nguyen

The *ganguro* phenomenon induced a peak in tanning salons and self-tanned products in Japan to achieve the heavy tanned look. The tanning business was blooming during this period in Japan. These UV-seeking activities undoubtedly play an important role in the higher risk of getting skin cancer. Many girls who followed the *ganguro* trend in the past now fear the consequences of heavy tanning. Regardless of the increased tanning practice during *ganguro* phenomenon, according to Dr.

Henry W. Lim, chairman of the Department of Dermatology for the Henry Ford Health System and immediate past vice president of the American Academy of Dermatology, tanning is far less culturally acceptable in Asia countries such as Japan⁴. It's even believed to be associated with a lower socio-economic status, says Lim.

MATERIALS AND METHODS

This research is under review of Institutional Review Board (IRB). It is filed under Exempted category where research posed no more than minimal risk to participants (See Index)

Population and sample size of interest

The target populations are Japanese American living in Oregon and Akita prefecture population, Japan. The sample sizes are Japanese American students at OSU and Japanese students at Akita International University. Total number of response is 40 with 20 participants from each sample sizes.

Survey questions

A survey consisted of 22 questions was made available to Japanese American students at Oregon State University and Japanese students at Akita International University (See Index 1 for complete survey). Questions were designed to investigate the behaviors toward skin cancer prevention through the use of various protection means (hat, umbrella, SPF-contained products). Additionally, survey questions also examined attitude toward lighter or darker skin as well as the practice of tanning. Surveymonkey.com was used as portal to collect response. Direct link to survey is: <http://www.surveymonkey.com/VananhIDresearchsurvey>

Analysis

Data collected was calculated with response count and response percent within the sample. Each question was analyzed by means of comparison in responses between Japanese American and Japanese participants. Because the measurements of all variables were categorical, appropriate p-values were calculated to compare two samples using Chi-square test statistics. All analyses were performed using Microsoft Excel and Minitab (version 15.1).

RESULTS

Participants report

	Male	Female	Total
OSU Participants	10	10	20
AIU Participants	8	12	20
Total	20	20	40

Table 3:Number of participants responded to survey

*note: one female participant from OSU skipped questions 5-21.

Current skin color

The survey showed that half of Japanese American participants have light neutral (20%) and light beige (30%). The other half, majority have darker skin tone ranging from medium beige to chestnut (40%) with percentage of 10% indicated a very light skin tone (Fair-slightly yellow base and very fair) (Figure 7). The extreme contrast in skin tone ranged from chestnut to very fair. In comparison, Japanese participants at AIU have quite an even skin tone selection from golden bronze to fair-slightly yellow base (Figure 7).

Regardless of the extreme spectrum in current skin tone, Japanese American participants in general wish to have a more ‘in between’ skin tones from medium beige to light beige (55%). The contrast in preference can be seen with the majority of Japanese participants preferred 2-3 lighter shades for skin tones, from apricot to very fair (73%). However,

there were two Japanese participants or 6% preferred very dark skin tone (bronze or espresso) (Figure 8). They were both male.

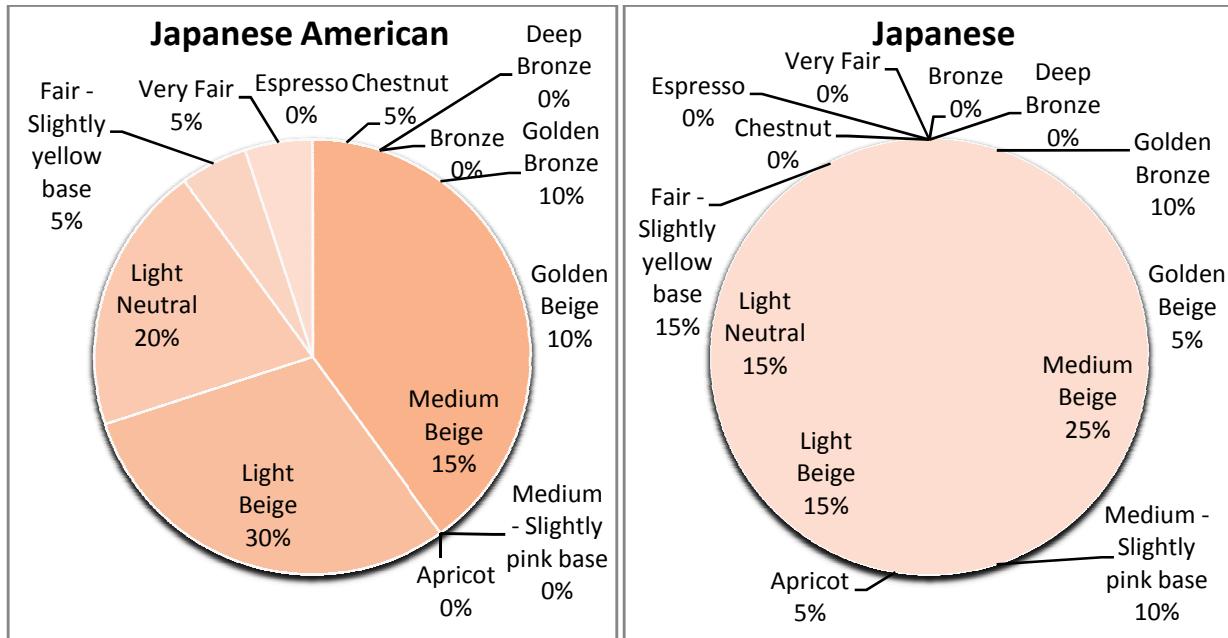


Figure 7: Current skin tone of OSU participants (Left) and AIU participants (Right)

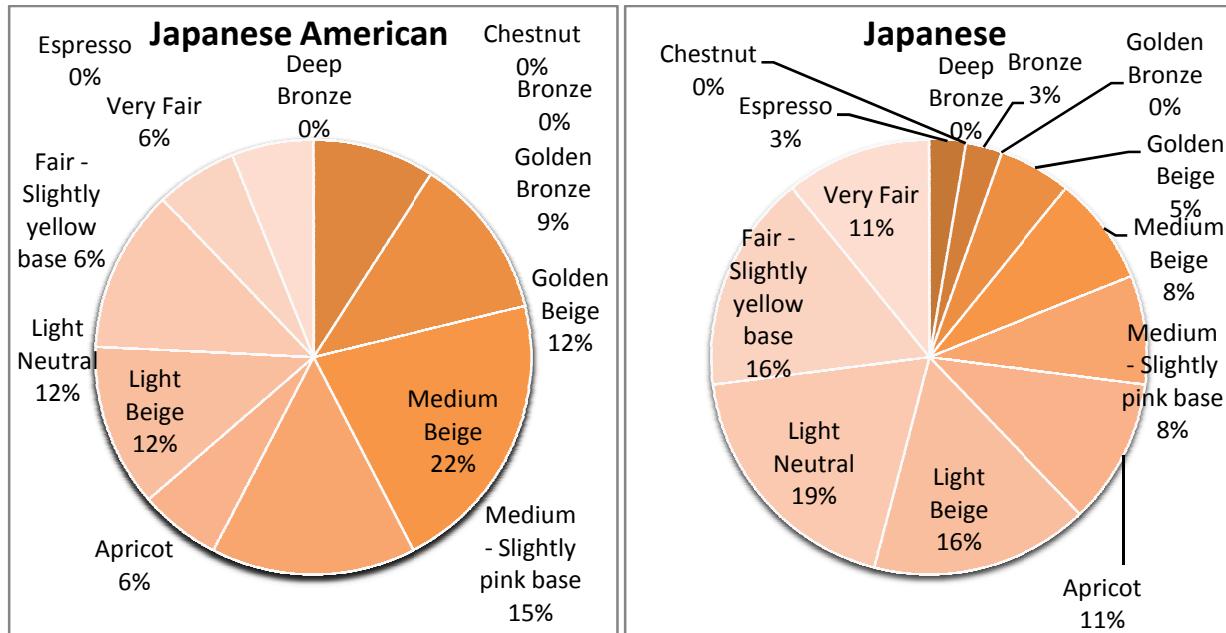


Figure 8: Skin tone preference of Japanese American (Right) and Japanese (Left)

The use of SPF-contained products

The majority of participants in both samples use sunscreen product either when they are outside or when it is really sunny or knowing that they have to stay outside for a long time, with the exception of 15.8% or 3 participants from OSU did not use sunscreen at all.

Surprisingly, more Japanese American participants indicated some sort of reapplication during the day than Japanese (Figure 9 and10). 57.9% Japanese American reapplies sunscreen while only 35% Japanese do so. Out of these numbers, 60% Japanese American female participants indicated some sorts of reapplication comparing with 50% Japanese female.

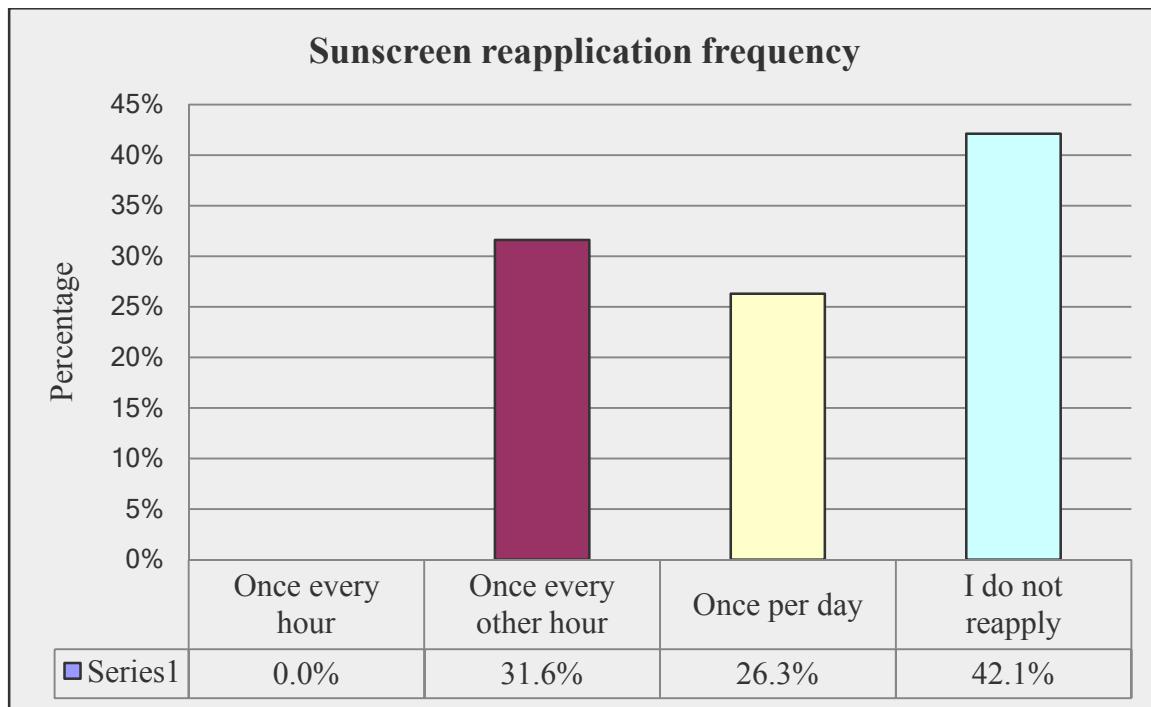


Figure 9: Sunscreen reapplication frequency from Japanese American participants

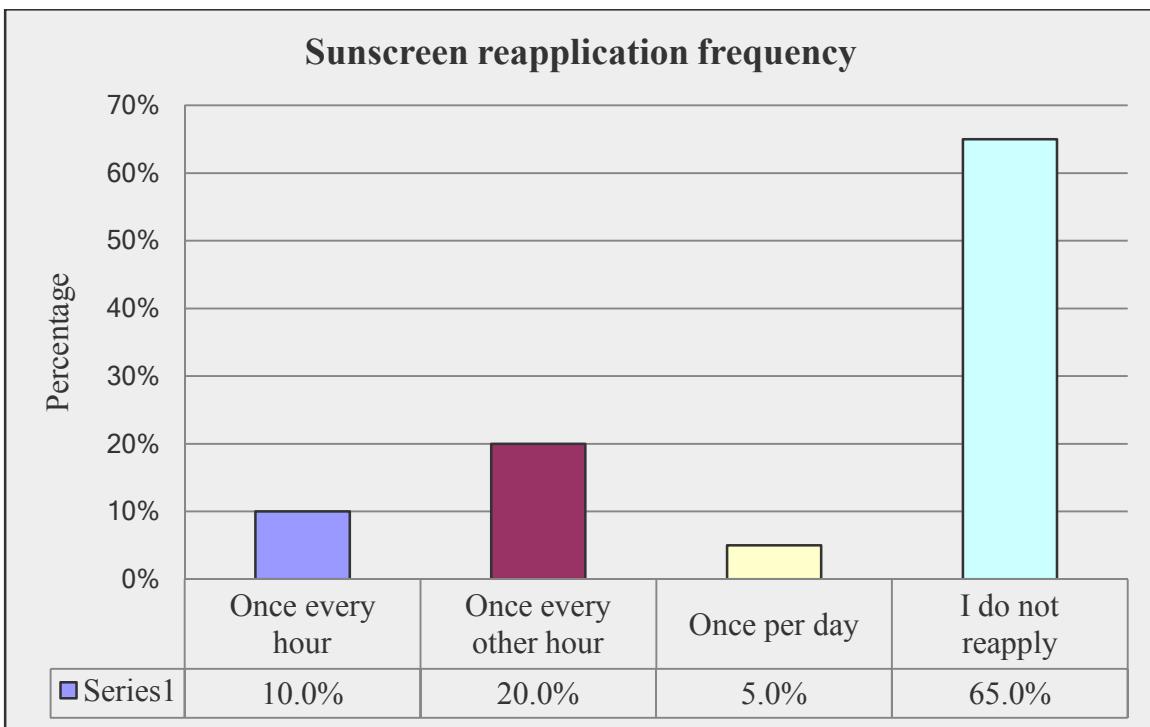


Figure 10: Sunscreen reapplication frequency from Japanese participants

Participants were also asked to indicate the occasions when they use SPF-contained product. The results between two sample sizes were somewhat similar (Figure 11 and12). Participants who picked option “Other” were asked to specify. There is a significant difference in answer for this option. 3 out of 18 participants from OSU (2 people skipped this question for unknown reason) chose this option indicated that they only use SPF-contained products in the summer only (coming from female participants) or when they are outdoors for a long period of time (male participant). Out of 6 Japanese survey takers or 30% who chose this option, 100% indicated that they always, all the time or use SPF-contained products in their everyday makeup routine. All of these participants were female.

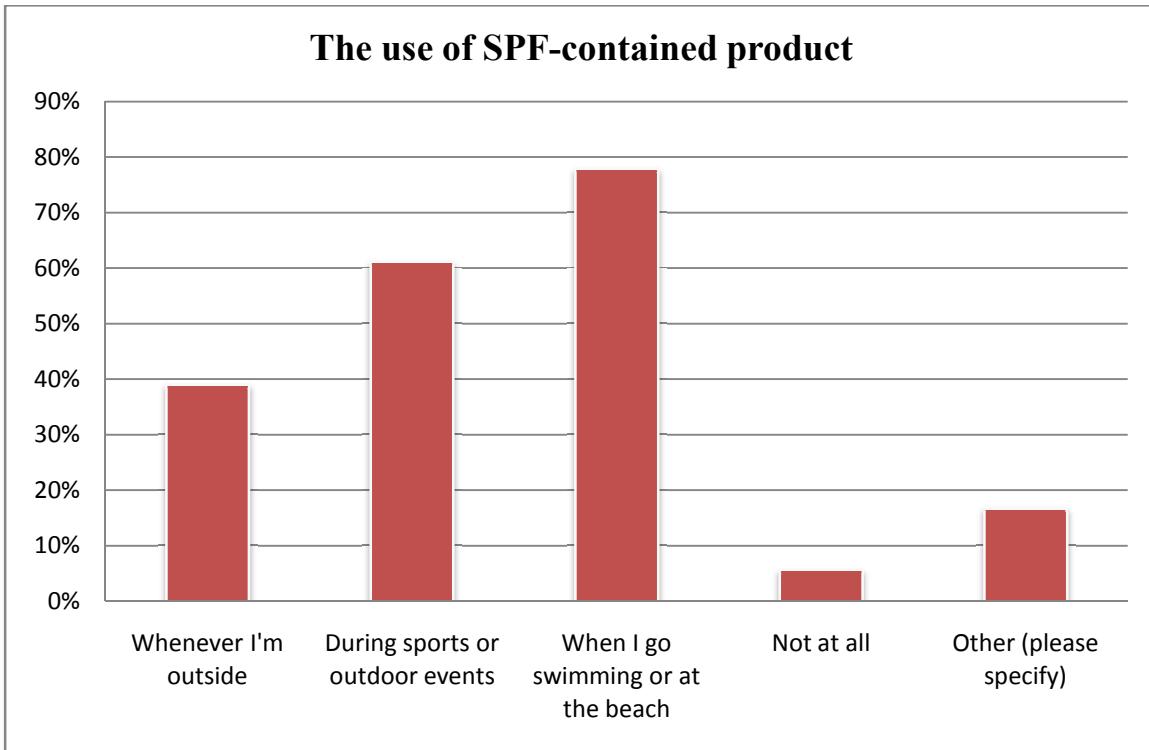


Figure 11: The use of SPF-contained product when outside from Japanese American participants

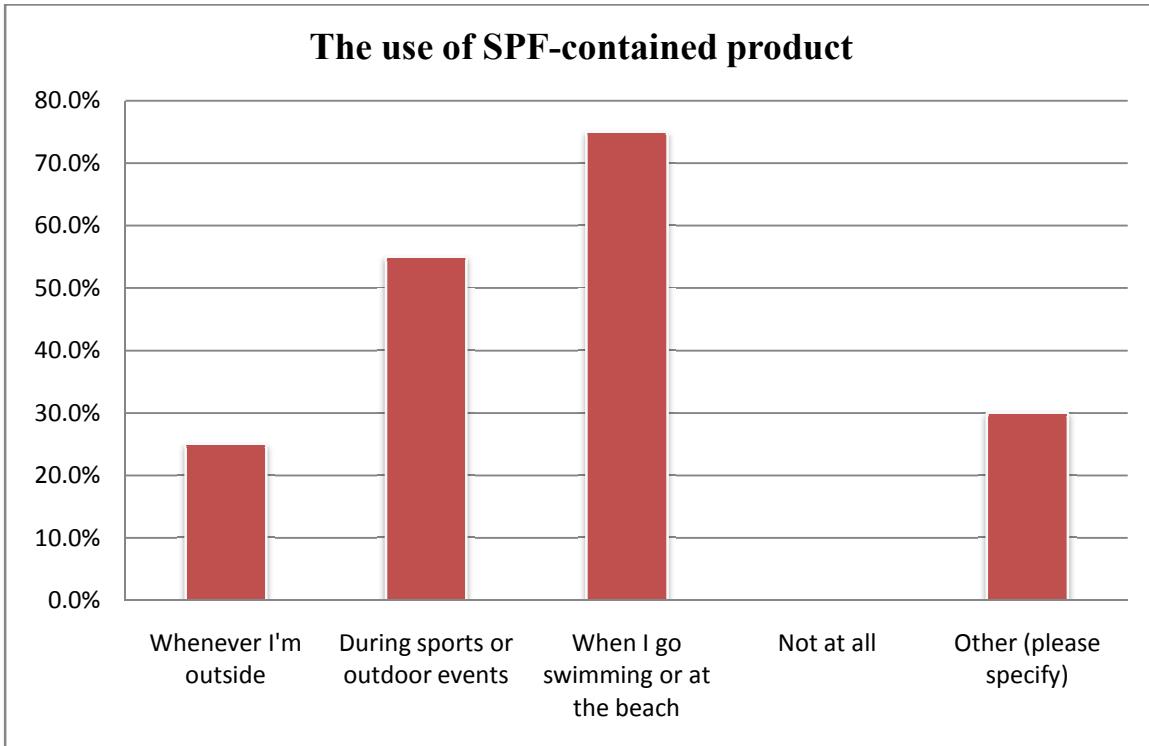


Figure 12: The use of SPF-contained product when outside from Japanese participants

Contrary to the belief, when asked about the intensity of SPF in SPF-contained products that they most often use, Japanese American and Japanese shared similar results (Figure 13). Majority use SPF 45 and below regularly. While there are more Japanese American using products contain SPF above 50, 11% also do not use any, comparing with 5% Japanese. Japanese also use more SPF 50 products (15% compared to zero from Japanese American participants).

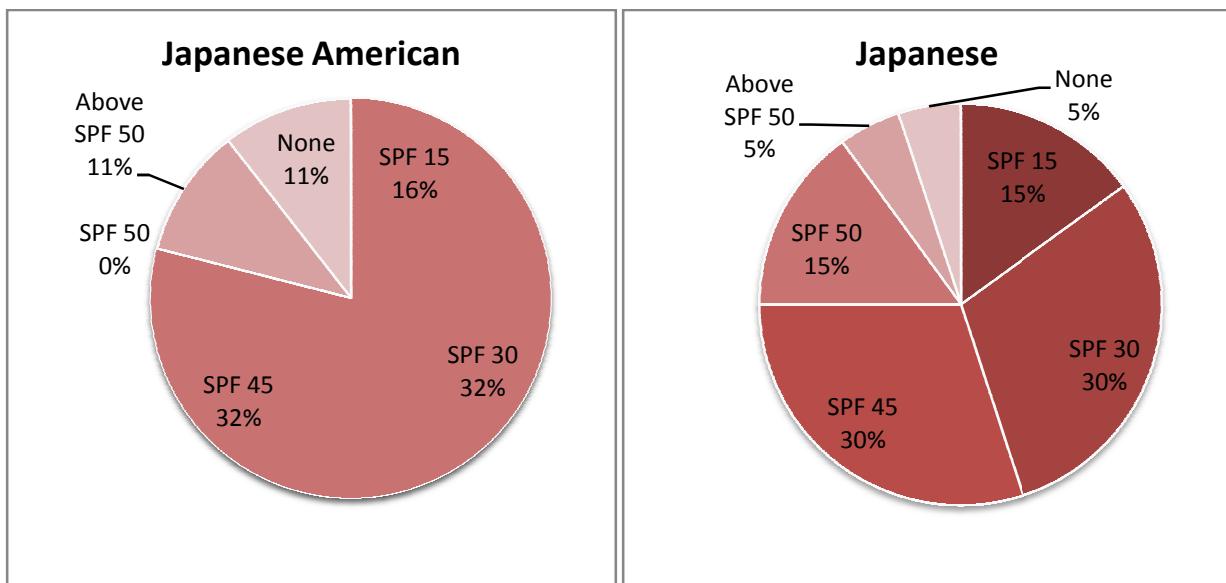


Figure 13: Type of SPF product most often used by Japanese American (Left) and Japanese (Right)

The use of hat and umbrella

Hat usage was not a very popular choice in Japanese American to shield the sun. 52.6% or 10 participants picked 'No' and only one male participant, made up of 10.5% of total, chose 'Yes'. In contrary, they majority of Japanese would wear hat in sunny weather with 45% of participants clicked the 'Yes' option and 15% only wear it when it is really sunny

or they have to stay outside for a long time. Male survey takers also made up of the majority of those that indicated the use of hat (6 out of 9).

Not surprisingly, the use of umbrella when sunny was even less of a popular choice in Japanese American (Table 4). Only one Japanese American female indicated umbrella practice when it is really sunny or knowing that she has to be outside for a long time. Six Japanese participants showed the usage of umbrella. Five of which was female.

	Japanese American	Japanese
Yes	0	2
No	18	14
Only when it's really sunny or have to be outside for a long time	1	4

Table 4: The use of umbrella under sunny condition

Skin whitening vs. tanning

Skin whitening usage is not a common choice from most Japanese American as shown in the survey results (Figure 14). 84.2% do not use skin whitening product at all and 10.5% indicated an occasional usage. Interestingly, one participant from OSU told us of the everyday usage. More surprisingly, this participant was male.

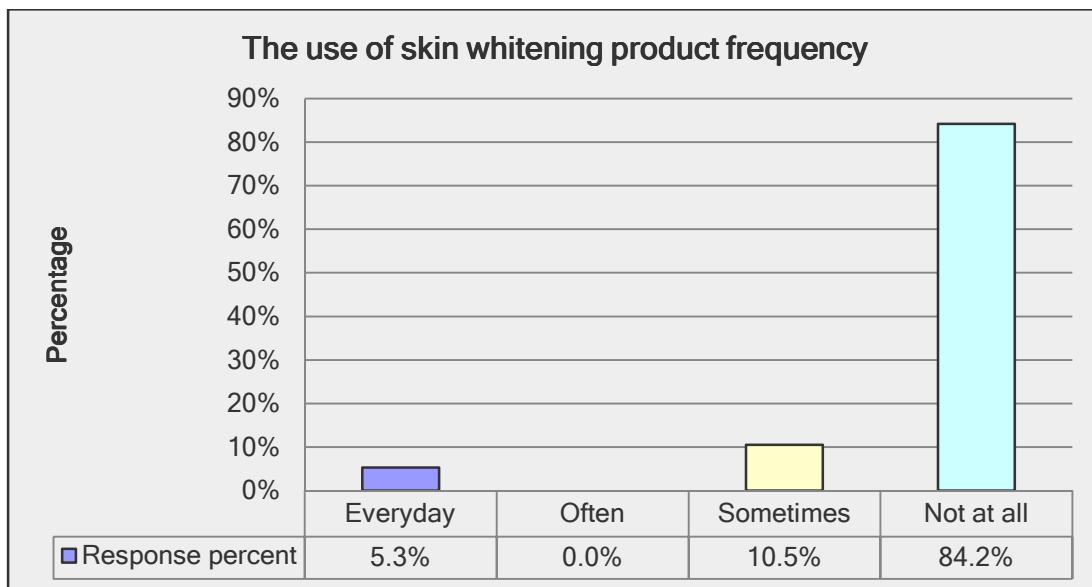


Figure 14: The use of skin whitening product frequency in Japanese American

The number of skin whitening products in Japanese sample size was as expected with 60% indicated some usage (20% chose ‘Often’ and 40% ‘Sometimes’). Out of the 40% that chose option ‘Sometimes’, 37.5% or three participants were male.

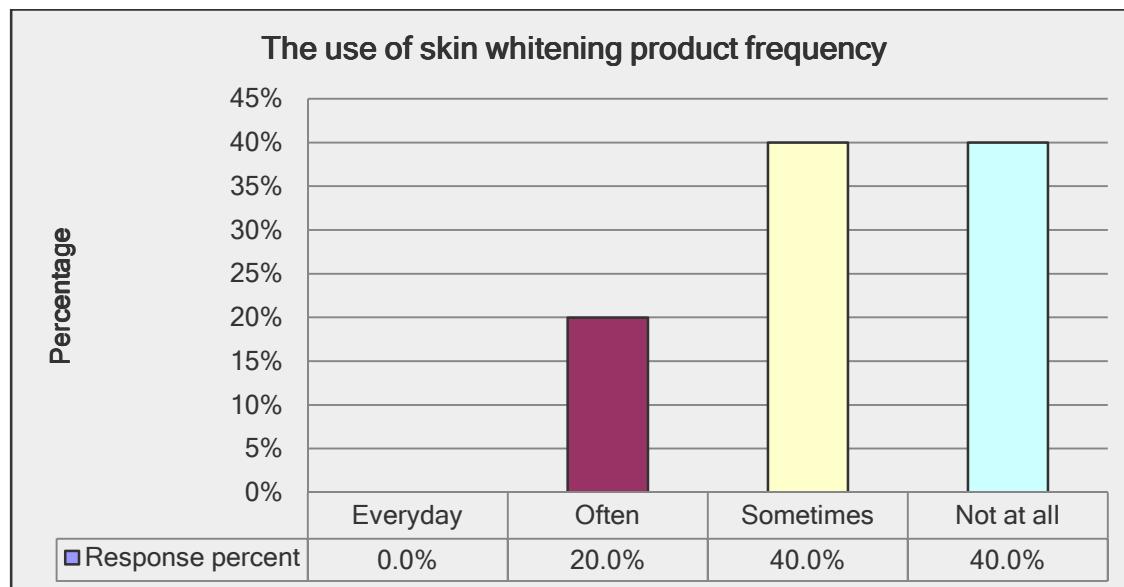


Figure 15: The use of skin whitening product frequency in Japanese

Survey result for tanning product use was most interestingly. While 95% of Japanese participants do not use tanning products (Figure 15), one male indicated that he sometimes uses them. Japanese participants do not go to tanning salon.

The frequency of tanning products usage in Japanese American is summarized in table 5 below. 36.8% indicated the use of tanning products occasionally were all female. However, participant who indicated the use of tanning product everyday was male. Additionally, out of five survey takers who go to tanning salon, one was male and this individual tan in winter times only. Except for one individual indicated a rare visit to tanning salon, the rest also tan only in winter times.

Frequency	Response percent	Response count
Everyday	5.3%	1
Often	0.0%	0
Sometimes	36.8%	7
Not at all	57.9%	11

Table 5: Frequency of tanning products usage in Japanese American

Opinion on tanned vs. white skin

Participants were asked to choose two answers on their opinion about tanned and white skin. On tanned skin, OSU participants think that it is healthy and pretty with 73.7% and 78.9% respectively. Interestingly, 85% of AIU participants think that tanned skin is

healthy but 60% also says it is not attractive. There is a significant difference in opinion on tanned skin between Japanese American and Japanese (p -value < 0.01) (Figure 13).

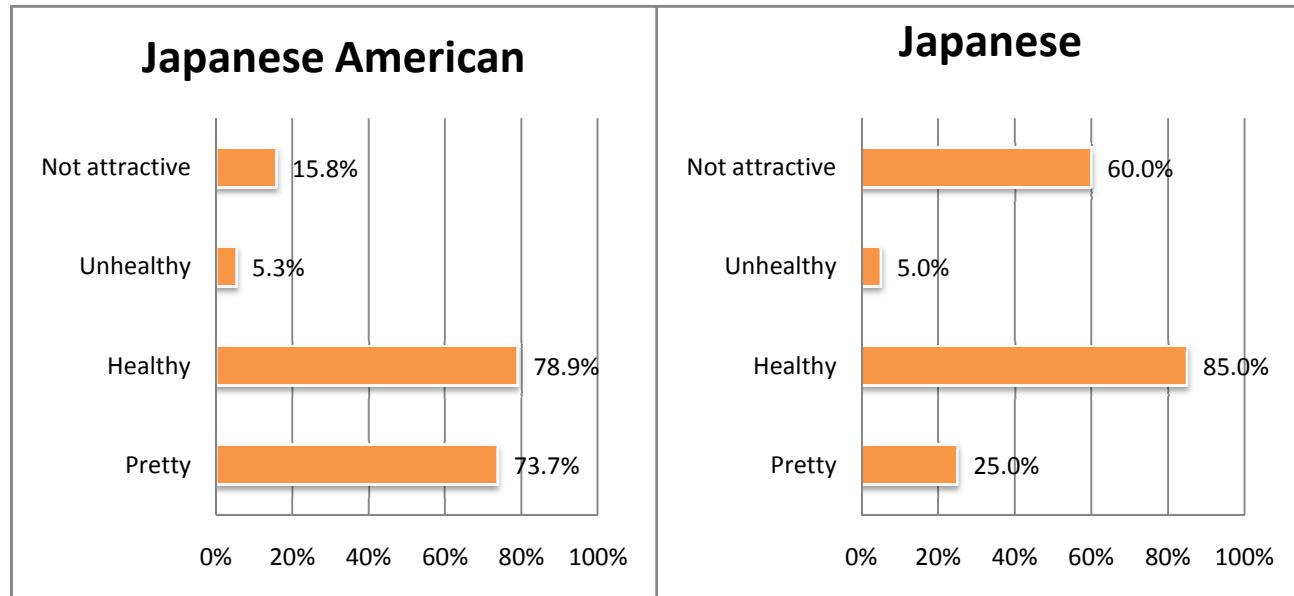


Figure 16: Opinion on tanned skin in Japanese American (Left) and Japanese (Right)

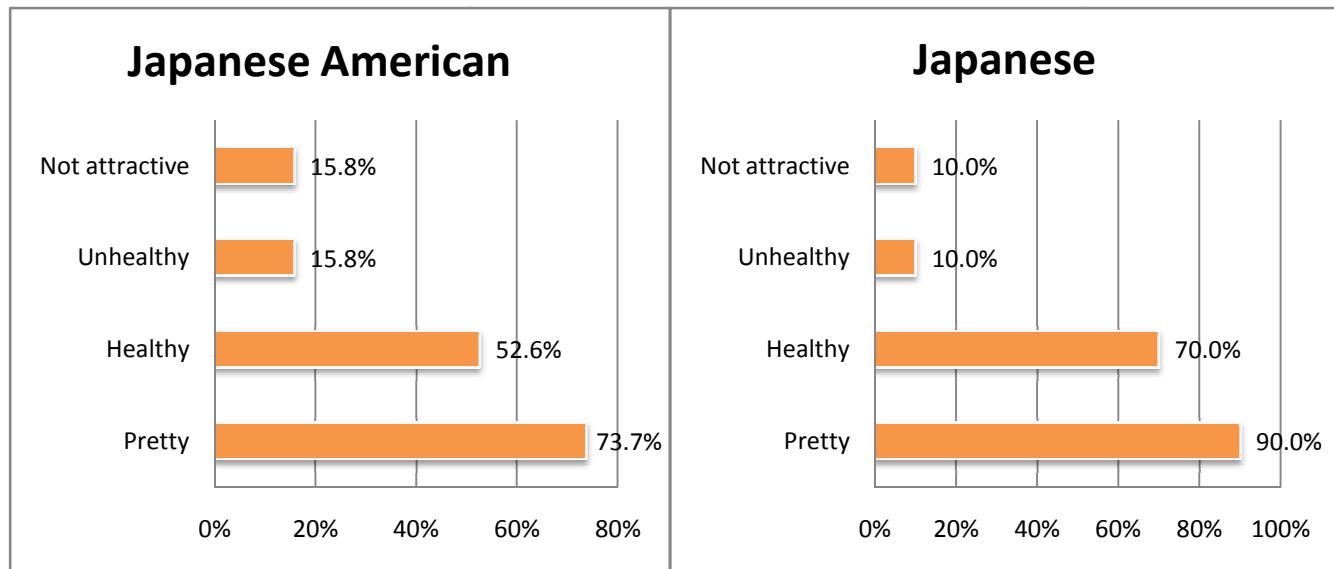


Figure 17: Opinion on white skin in Japanese American (Left) and Japanese (Right)

When asked about opinion on white skin, Japanese American and Japanese shared similar answers (Figure 16). There was not a significant difference in responses (p -value > 0.01).

The majority thinks that light skin color is both healthy and pretty. There is not a difference in gender in answer choice in this particular question.

Skin cancer perception

Survey takers were asked to provide opinion on the skin cancer rate among Asian and Asian American. Results are as below:

	Japanese American	Japanese
It's decreasing	0.0%	5.0%
It's increasing	21.1%	35.0%
It's not a problem among Asian	78.9%	55.0%
Other (please specify)	0.0%	5.0%

Table 6: Perception on skin cancer rate among Asian

	Japanese American	Japanese
It's decreasing	0.0%	0.0%
It's increasing	63.2%	65.0%
It's not a problem among Asian	36.8%	30.0%
Other (please specify)	0.0%	5.0%

Table 7: Perception on skin cancer rate among Asian American

Most Japanese American thinks that skin cancer is not a problem among Asian (78.9%).

While there are less Japanese believe in the irrelevance of this disease among Asian (55%), one participant think that it is actually increasing and another specify the lack of knowledge in this matter. However, when asked about skin cancer rate among Asian American, majority of both Japanese American and Japanese participants think that it is on the rise as 63.2% and 65%, respectively, indicated in the answer choice.

Participants were also asked to write-in answer for one skin disease that they think is most prevalent to them and one skin disease that they are most afraid of. Raw answers are shown in table 8 and 9. There was an equal number of cancer or melanoma as most prevalent skin disease to participants between Japanese American and Japanese.

Interestingly, while there was four Japanese American participants indicated skin cancer incidence in family, none of these participants think that cancer is most prevalent skin disease to them. The only Japanese survey taker that had skin cancer incidence in family believes that melanoma is most prevalent to her.

Not surprisingly cancer or melanoma was the most feared skin disease among Japanese American and Japanese. Other skin diseases that are dreaded include flesh eating diseases, acne, rosacea, eczema and shingles.

Number	Prevalent skin disease	Skin disease most afraid of
1	Eczema	Melanoma
2	Psoriasis	Melanoma
3	Cancer	Cancer
4	Psoriasis	Cancer
5	Acne	Melanoma
6	Eczema	Melanoma
7	Acne	skin cancer
8	acne	skin cancer

9	acne	flesh eating diseases
10	Acne	Metastatic melanoma
11	Acne	Acne
12	eczema	skin cancer
13	Eczema.	Skin cancer.
14	cancer	Cancer
15	skin tanned	skin cancer
16	Melanoma	Melanoma
17	Sclerosis	Any flesh eating disease
18	Skin cancer (melanoma?)	Skin cancer
19	Melanoma	Acne

Table 8: Perception of prevalent and most feared skin disease from Japanese American

Number	Prevalent skin disease	Skin disease most afraid of
1	psoriasis	rosacea
2	rashes	skin cancer
3	acne	cancer
4	skin cancer	skin cancer
5	rash	skin cancer
6	Acne	Eczema
7	Eczema	Rosacea
8	eczema	melanoma
9	skin cancer	skin cancer
10	Pimple	Skin cancer
11	acne	skin cancer
12	atopy	shingles
13	Acne	Eczema
14	dry skin	skin cancer
15	Atopy	Melanoma
16	skincacer	skin cancer
17	skin cancer	skin cancer
18	acne	skin cancer
19	I am not sure about this	I am not sure about this
20	Cancer	Cancer

Table 9: Perception of prevalent and most feared skin disease from Japanese

DISCUSSION

While fair skin has been known as a risk factor in skin cancer development among Japanese population who has the lightest natural skin tone comparing to other Asian ethnicities⁴,

Japanese are still actively seeking fairer complexion through various pain-staking means. This irony lies in the social, cultural norms that are embedded in Japanese concepts of beauty.

Beauty value generates societal and behavioral attitudes. Since white skin preference is an important aspect valued not just as an esthetic matter but also as an indicator of social class, skin care practice is prioritized in beauty care in Japan. As a result, certain behaviors in skin cancer prevention practices found in native Japanese are different comparing to Japanese living in other Western countries such as America.

The purpose of this survey research was to identify the differences societal and behavioral attitudes toward skin cancer between Japanese American and Japanese. Skin tone preference, the usage of SPF-contained products, hat and umbrella, skin whitening and tanning products, opinion on white and tanned skin and perceptions on skin cancer were used as premises for comparison.

Differences were found for skin tone preference where Japanese American wished to have a more middle skin tones from medium beige to light beige while majority of Japanese preferred 2-3 shades lighter for skin tones, from apricot to very fair. There was also a distinctive viewpoint toward tanned skin. While the majority of Japanese thought that tanned skin was healthy, they did not think it was attractive, at least not anymore.

Different in opinion on tanned skin and skin tone preference, Japanese are more likely to go further to achieve lighter complexion than Japanese American, as exhibited in their pain-taking effort of using umbrella the shield from the sun or the use of skin whitening products. While there are some distinct differences in the practice of using umbrella, whitening and tanning skin products, there is not a significant difference in the use of SPF-contained products. Additionally, contrary to the neutral opinions from Japanese American, the majority of Japanese

view fondly of white skin over tanned skin. Both populations also share similar perceptions about skin cancer as not a problem among Asian but an increasing issue in Asian American.

There are limited researches on differences of skin cancer prevention practices between Japanese and Japanese American that might be the stem of the disparity in skin cancer rate between these two populations. Understanding cultural effects on skin protection and prevention has a vitally important role in public health promotion and prevention of this deadly disease.

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December 3, 2010

Dear Oregon State University's Institutional Review Board committee,

This is to support Ms. Vananh Nguyen, undergraduate senior in Bioresource Research and International Degree (ID) program, regarding her senior research project in ID. Akita International University is willing to be a support site and liaison in the dissemination of the survey link to AIU's students as Ms. Nguyen requested.

Sincerely,



Daisuke Machida
Executive Officer

INFORMED CONSENT FORM

Differences in societal and behavioral attitudes toward skin cancer between Japanese American and Japanese

Principal Investigator: Dr. Nancy Rosenberger

Student Researcher: Vananh Nguyen

Co-Investigator(s):

OSU's Department of Anthropology and AIU's Department of External Affairs

Version Date: 09/30/2010

1. WHAT IS THE PURPOSE OF THIS FORM?

This form contains information you will need to help you decide whether to be in this study or not. Please read the form carefully and ask the study team member(s) questions about anything that is not clear.

2. WHY IS THIS STUDY BEING DONE?

The purpose of this study is to investigate the contribution of cultural behaviors and societal attitudes toward skin cancer diseases.

The study is being conducted for the completion of thesis for the Baccalaureate of Arts in International Studies program.

Up to 100 Japanese students from Oregon State University and Akita International University will be invited to take part in this study.

3. WHY AM I BEING INVITED TO TAKE PART IN THIS STUDY?

You are being invited to take part in this study because you identified yourself as a Japanese American or a native Japanese currently living in Japan.

4. WHAT WILL HAPPEN IF I TAKE PART IN THIS RESEARCH STUDY?

You will be given a link to an online survey consists of 10 questions written in English. The majority of the questions are in the form of multiple choices, others including inserting numerical values as well as written responses. Data will be collected anonymously and analyzed based on your responses.

Study duration: from September 15th 2010 – December 15th 2010

Future contact: We may contact you in the future for another similar study. You may ask us to stop contacting you at any time.

Study Results: The study results will be compiled in the undergraduate thesis archive that is available at Oregon State University's Library Archives at <http://osulibrary.oregonstate.edu/>

5. WHAT ARE THE RISKS AND POSSIBLE DISCOMFORTS OF THIS STUDY?

There are no foreseeable risks to participating to this study. It is in a form of online survey and will be collected anonymously.

You may experience side effects from the study procedures that are not yet known to the researchers such as:

Internet: The security and confidentiality of information collected from you online cannot be guaranteed. Information collected online can be intercepted, corrupted, lost, destroyed, arrive late or incomplete, or contain viruses.

6. WHAT ARE THE BENEFITS OF THIS STUDY?

This study is not designed to benefit you directly. However, the result for this project will contribute to the understanding of cultural effects on skin protection and prevention. It has a potentially important role in public health promotion and prevention in America.

7. WILL I BE PAID FOR BEING IN THIS STUDY?

You will not be paid for being in this research study.

8. WHO WILL SEE THE INFORMATION I GIVE?

The information you provide during this research study will be kept confidential to the extent permitted by law. Research records will be stored securely and only researchers will have access to the records. Federal regulatory agencies and the Oregon State University Institutional Review Board (a committee that reviews and approves research studies) may inspect and copy

records pertaining to this research. Some of these records could contain information that personally identifies you.

If the results of this project are published your identity will not be made public.

To help ensure confidentiality, we will not ask for any personal information on the survey, other than identification of the school you are enrolled in. Other information from data are collected anonymously

9. WHAT OTHER CHOICES DO I HAVE IF I DO NOT TAKE PART IN THIS STUDY?

Participation in this study is voluntary. If you decide to participate, you are free to withdraw at any time without penalty. You will not be treated differently if you decide to stop taking part in the study. If you choose to withdraw from this project before it ends, the researchers may keep information collected about you and this information may be included in study reports.

10. WHO DO I CONTACT IF I HAVE QUESTIONS?

If you have any questions about this research project, please contact:

Dr. Nancy Rosenberger
(541) 737-3857
nrosenberger@oregonstate.edu

Vananh Nguyen
(503) 415-0134
nguyenvna@onid.orst.edu

If you have questions about your rights or welfare as a participant, please contact the Oregon State University Institutional Review Board (IRB) Office, at (541) 737-8008 or by email at
IRB@oregonstate.edu

Your review of this letter indicates that this research study has been explained to you, that your questions have been answered, and that you agree to take part in this study.

SURVEY QUESTIONS
www.surveymonkey.com/VananhIDresearch

PAGE 1

1. Which university do you go to?

- Akita International University
- Oregon State University

2. What is your sex?

- Male
- Female

3. What is your current skin tone?

Please go to http://sphotos.ak.fbcdn.net/hphotos-aksnc4/hs838.snc4/69820_714836290378_19715913_39896739_2823962_n.jpg for visual picture of skin tone

- Very Fair
- Fair - Slightly yellow base
- Light Neutral
- Light Beige
- Apricot
- Medium - Slightly pink base
- Medium Beige
- Golden Beige
- Golden Bronze
- Bronze
- Deep Bronze
- Chestnut
- Espresso

4. What is your preferred skin tone? (please choose 2)

Please go to http://sphotos.ak.fbcdn.net/hphotos-aksnc4/hs838.snc4/69820_714836290378_19715913_39896739_2823962_n.jpg for visual picture of skin tone

- Very Fair
- Fair - Slightly yellow base
- Light Neutral
- Light Beige
- Apricot
- Medium - Slightly pink base
- Medium Beige
- Golden Beige
- Golden Bronze
- Bronze
- Deep Bronze

- Chestnut
- Espresso

PAGE 2

1. Do you often use SPF-contained products when you are outside?

- Yes
- No

2. How often do you reapply sunscreen when you are outside?

- Only when it's really sunny or I have to stay outside for a long time
- Once every hour
- Once every other hour
- Once per day
- I do not reapply

3. When do you use SPF-contained products? Check all that apply

- Whenever I'm outside
- During sports or outdoor events
- when I go swimming or at the beach
- Not at all
- Other (please specify)

4. What is the type of SPF product that you most often use?

- SPF 15
- SPF 30
- SPF 45
- SPF 50
- Above SPF 50
- None

5. Do you often wear a hat when you are outside?

- Yes
- No
- Only when it's really sunny or I have to be outside for a long time

6. Do you use an umbrella when it's sunny?

- Yes
- No
- Only when it's really sunny or I have to be outside for a long time

PAGE 3

1. How often do you use skin whitening product?

- Everyday
- Often

- Sometimes
- Not at all

2. How often do you use tanning products?

- Everyday
- Often
- Sometimes
- Not at all

3. Do you go to tanning salon?

- Yes
- No

4. If you click yes for question above, how often do you tan at a tanning salon?

- Often (regardless of season)
- Winter times only
- Sometimes
- Rarely

5. To you, tanned skin is: (check 2 that apply)

- Pretty
- Healthy
- Unhealthy
- Not attracted

6. To you, white skin is: (check 2 that apply)

- Pretty
- Healthy
- Unhealthy
- Not attracted

7. Is there anyone in your family who has skin cancer? (Check all that apply)

- No one
- Parents
- Grandparents
- Siblings
- Other relatives (please specify)

8. What do you think about skin cancer rate among Asian?

- It's decreasing
- It's increasing
- It's not a problem among Asian
- Other (please specify)

9. What do you think about skin cancer rate among Asian Americans?

- It's decreasing
- It's increasing

- It's not a problem among Asian American
- Other (please specify)

10. What is one skin disease that you think is most prevalent to you? (Write answer)

11. What is one skin disease that you are most afraid of? (Write answer)