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作品名稱　**UVB induced TRPV1 and TRPA1 expression in skin keratinocyte and dorsal root ganglion cells: a plausible cause of warm and pain by sunlight irradiation**

得獎獎項　**三等獎**

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關鍵詞　**TRP、皮膚角質細胞、背根神經元**

作者簡介

Hi everyone, my name is Madison. I am from Kaohsiung. I have always been enthusiastic about digging questions and finding out the resolution. Therefore, I love conducting experiments to discover new things. My favorite subject is biology, as I’ m interested in animals and creatures. Besides my academic performance, I am interested in hiking and swimming. As for hiking, I had the opportunity to climb up Mount Fuji. My companions and I helped each other along the way and successfully reached the summit. To be able to witness the sunrise on top of Mount Fuji was a once-in-a-lifetime adventure. My strongest strengths are perseverance and empathy. I participated in the biology fair. Although we faced numerous obstacles in pursuing the research aims and spent hours in finding the right solution, I learned to never give up and find different ways to fix problems.

Abstract

People found themselves to feel tingling pain immediately after excessive sun exposure before the skin becomes inflamed and red. This defense mechanism is to help people to avoid excessive sun exposure. Why people would sense the pain sensation after sun exposure? Previous literatures investigating the light receptor in human body focused on the opsin 1/2 in the cone and rod cells in eye retina or the opsin 3 in skin. However, the expression of these opsins is limited in dorsal root ganglion cells (DRG cells), which is the principal cells in the skin to sense the environmental stimuli in the skin nerve endings. Transient receptor potential cation channels (TRP channels) are the other possible photoceptors in skin. TRP channels are expressed robustly in skin and nerves. Among them, TRPV1 and TRPA1 are expressed in epidermal keratinocytes, melanocytes, and dermal fibroblasts with different physiological functions. Dr. David Julius was awarded the 2021 Nobel Laurette for physiology. TRPV1/TRPA1could be activated by temperature, acidity, and chemicals, however, whether UVB would induce TRPV1 and TRPA1 expression and functional calcium propagation in DRG cells and keratinocytes remain unknown. I irradiated human epidermal keratinocytes and rat DRG cells with different intensity of UVB and measured the protein expression of TRPV1 and TRPA1 using flow cytometry and immunofluorescent microscope. The calcium signals were measured dynamically using calcium imaging in Flou-4-incubated cells. The result showed that UVB at 10 mJ/cm2 induced a modest protein expression of TRPA1 and TRPV1 in DRG cells. However, UVB irradiation at 5 mJ/cm2 induced a significant expression of TRPA1 but not TRPV1 in DRG cells. For keratinocytes, UVB irradiation at 10 mJ/cm2 induced significant expression of both TRPV1 and TRPA1 (for TRPA1 in particular) in keratinocytes, however, UVB irradiation at intensity higher than 20 mJ/cm2 induced cell toxicity in keratinocytes. The calcium propagation in keratinocytes seems similar when they are irradiated with UVB. This pivotal study showed that UVB indued the expression of TRPV1 and TRPA1 in particular in both keratinocyte and DRG cells, which might be associated with the pain elicited by sunlight.

摘要

陽光晒到皮膚，人體會感覺到刺痛以避免過強紫外線的曝露。但皮膚為何會有刺痛的感覺呢?過去對光的生體受器著重於眼睛錐及桿狀細胞(Opsin 1/2)，但它們在背根神經元(DRG，周邊神經末端主體細胞)的表現並不多。TRP channels 表現在皮膚及神經。TRPV1 是一種痛覺受器，活化時會有鈣離子通透，Dr. Julius 因它得到 2021 諾貝爾獎。TRPV1/A1 可受溫度、酸度等活化，但 DRG 細胞表現的 TRPV1/A1，是否會因紫外線(UVB)照射而影響，並導致鈣通透，目前並不清楚。我以不同強度 UVB 照射人類角質細胞或大鼠 DRG，以螢光顯微鏡及流氏細胞儀測量 TRPV1/A1 蛋白質表現，以實時影像來作動態鈣離子分析。結果顯示 UVB 在 10 mJ/cm2 可增加 DRG 的 TPRA1/V1 表現，但 UVB 在 5mJ/cm2 照射 DRG 細胞後，只增加 TRPA1 而不是 TPRV1 的表現。在皮膚的角質細胞，不管是使用螢光顯微鏡或是流氏細胞儀，UVB 在 20mj/cm2 以上的能量強度會造成角質細胞的毒性，以 10mJ/cm2 UVB 照射角質細胞，則會增加角質細胞的 TPRA1 及 TRPV1 表現，其中又以 TRPA1 的增加較明顯。惟 UVB 照射對細胞鈣離子通透的實時影響不大。我的結論是，UVB 照射增加角質細胞及 DRG 的 TRPA1/V1 表現(特別是 TRPA1)，這些改變可能與光照引起之麻痛有關。

1. Motive

In class of physics regarding thermal engineering, the teacher in high school told us the heat transfer could be based on various mechanisms, including thermal conduction, thermal convection, thermal radiation, and a recent theory of energy transfer by phase changes. The thermal radiation is mediated by photons and occurs across vacuum. The thermal irradiation from the Sun is transported to the Earth in the solar system. The solar irradiation brings the heat and the energy that necessitate the development, homeostasis, and even the revolution, of the individual animals, plants, and ecosystems. The heat from the solar irradiation is carried mainly by the infrared spectrum of the electromagnetic irradiation. However, this heat transfer is mainly exchanged among the two physical objects. As a biological and a live organism that belong to homeotherms to receive heat irradiation, our biological body system has a delicate heat homeostasis system that maintains the body temperature through perspiration and vascular dilatations for heat dissipation. Moreover, our human body could sense several exogenous stimulators. The physical touch and pain stimulation activate the nerve endings in the skin and the signal is transmitted to spinal cord and brain for an individual human to recognize and interpret the feeling. Likewise, the photons in solar irradiation could also potentially activate the nerve endings or nerve acceptors to generate and relay the electromagnetic pulse in the nerve system and eventually generate sensations in the brain.

When human body is irradiated by the solar irradiation, it senses the photons and electromagnetic impulses, which generate not only the warmth sensation, but also the tingling, burning, or prickling pain in the brain cortex. This pain sensation is in particular, accentuated during the noon time when the sunlight irradiation is most intense during the day. This pain sensation could be viewed as an alarm, to warn our body that the sun light irradiation is too excessive and dangerous, so that our body should avoid further sun light exposure. However, what is the mechanism by which the sun light result in the acute sensation of tingling, burning, or prickling pain in the human body? This demands further investigation.

According to the difference of the component electromagnetic waves regarding the wave length, the sunlight could be categorized by infrared, visible light, and ultraviolet irradiations. Infrared irradiation carries heat as a major cause of heat irradiation that might increase skin temperature, which indirectly causes warmth sensation. However, the human body has its own homeostasis system, which has the ability to blunt the elevated skin temperature by evaporation of sweats and vascular dilatation. Moreover, the actual energy carried by infrared irradiation, based on the Planck relation, is less than that by visible light or ultraviolet irradiation. Therefore, the direct nerve/acceptor stimulation effects on human skin could be less than other visible light or ultraviolet irradiation. On the other hand, ultraviolet irradiation, with low wave length and high energy, could

have more direct stimulation effects on the human skin. Taken together, I thought to determine how the ultraviolet irradiation, before causing skin redness and inflammation, results in the immediate tingling and prickling pain on the human skin. What are the mechanisms by which the peripheral nerve ending and/or acceptors are activated by the ultraviolet irradiation?

2. Purpose

I would like to investigate how the ultraviolet irradiation, before causing skin redness and inflammation, results in the immediate tingling and prickling pain on the human skin. What are the mechanisms by which the peripheral nerve ending and/or acceptors are activated by the ultraviolet irradiation?

3. Literature reviews

3.1.Potential photoceptors in animals

The intensity of the sunlight reaching Earth surface depends on a lot of factors, including altitude, latitude, season, and particle densities in the atmosphere. What are the know potential photoceptors in the human body? Based on the light-transparency in the human body, eyes and skin are the two main organs that could possible possess these photoceptors. Cytochrome c oxidase，G-protein-coupled receptor opsins (OPNs)，circadian transcriptional factors (CRYs) are known potential photoceptors in the human body[1].

The eye is a specialized organ that could sense the light, in particular, it senses the visible light. The photoceptors in the rod and cone cells in the retina are the main ones that people are familiar with. However, other than eyes, there are specialized skin photoceptors in non-primate animals. In particular, skin is the biggest body organ that separates the host body and the outside environment. Those chromatophores in the skin are present in the skin of fish, frog, crabs, and the cephalopods (such as squid and octopus). These chromatophores sense the environmental changes and either to mimic the body color with the environments to cheat the enemies, or to show the vibrant color to startle the enemies, or as epigamic coloration to attract the same kind with opposite sex[2]. There are also cryptochromes, that were used as the non-visual acceptors, including those found in the antennas and brain of insect or in the bird retina [3].

3.2. Opsin 3, a photoceptor

Scientific study has found that mammals express several opsins, including the opsin 1 and

opsin 2 in the retina (opsin 1: blue, green, and red con opsin; opsin 2: rhodopsin); opsin 4 (melanopsin, which is involved in pupil dilation and circadian rhythm. Human and mouse skin harbors many opsins, but the most abundant one is Opsin 3[1]. Opsin 3 is expressed in many skin cells, including epidermal keratinocytes, melanocytes, and dermal fibroblasts [4].

To date there are only very few studies that have investigated how opsin 3 is affected by light irradiation. Castellano et al. found that blue light irradiation on the skin keratinocytes affects their expression of opsin 3 and the cellular differentiation[5]. Regarding skin melanocytes, the role of opsin 3 in the transfer of melanosomes from melanocytes to keratinocytes remains unclear[6]. However, surprisingly opsin 3 is not present in the dorsal root ganglion (DRG) cells, which mainly sense the stimulations in the epidermis.

3.3.Transient receptor potential cation channels (TRP channels)

Other than opsins, the plausible photoceptor may include TRP channels. TRP channels are expressed in many cells. In the fruit fly study, the authors found that Drosophila without TRP would have reduced light sensitivity. TRP could be categorized into several groups, including TRP C (canonical), TRP V (vanilloid), TRP M (melastatin), TRP N (no mechanoreceptor potential C), and TRP A (Family A)[7].

TRPV1, also called capsaicin receptor, is the TRP channels that was first discovered and recognized. As the name implies, TRPV1 is the protein that mediates the heat-burning sensation when the skin is applied with capsaicin (from chili peppers). Dr. David Julius found that TRPV1 is a heat-activated channel that mediates pain sensation [8]. Because of the TRPV1 discovery, he was awarded for Nobel's Laurette in 2021 for Physiology and Medicine. Function of TRPV1 not only includes the heat and pain sensation but also involves in the body temperature regulation[8]. In the DRG, TPRV1 cooperates with TRPA1 [9] to sense the environmental stimuli[10]. TPRV1 is a non-selective cationic ion channel that could be activated by a variety of environmental stimuli, including temperature higher than 43 degrees in Celsius, acidic condition, chili pepper, ally isothiocyanate (the reason why Wasabi tastes spicy), and endogenous acidic stimuli, such as endocannabinoid anandamide, N-oleyl-dopamine, and N-arachidonoyl-dopamine. Dr. Weinkauf in Universität Heidelberg of Germany found that UV irradiation in human body enhances the expression of several mediators, including TRPV1 [11]. However, how the light or UV affect the skin sensation through TRPV1 remains unclear. One experiment showed UV(B) induces pain sensation through NGF (Nerve growth factor) excretion that accentuates the TPRV1 response[12]. Chizh in UK found that SB-705498, an inhibitor for TPRV1, decreased the UVB-induced pain hypersensitivity[13]. Schaffler et al. found that oral TRPV1 inhibitor recapitulates this finding in human[14]. Nevertheless, how UVB by itself induces skin pain sensation DIRECTLY remains unknown.

TRPA1, like TRPV1, is also a calcium channel protein which is present in DRG cells. Several mediators could induce TRPA1 activation and calcium propagation, including allyl isothiocyanate or acrolein. Hill et al. in Germany found that UVA1 irradiation induces oxidative stress in human embryonic kidney cells[15]. Again, the literatures regarding how UV regulates TRPA1 are limited. Guntur et al. in Duke University of USA found that UV irradiation induces H_2O_2 expression through TRPA1 in fruit fly[16]. In humans, UV irradiation induces TRPA1 expression in melanocytes[17], which produce melanin. To date, there are very few investigations looking into how UV affects TRPA1 in DRG cells.

3.4.The effect of UVB on the expression of TRP channels in skin cells

There are again limited numbers of studies looking into how UV affect TRP in other skin cells. Chueh et al. 闕小輝教授 in National Defense Medical Center in Taiwan found that UVB induces induced Nrf2 degradation via TRPV1 in human skin fibroblasts. [18] Consistently, the same team found that UVB regulates the expression of cytokeratin 1/1 through TRPV1. [19] Chung et al. in Seoul University in Korea found that UVB induced gasdermin activation in keratinocytes through TRPV1 [20]. Recently in 2021, Camponogara et al. found that TRPV1 inhibitor could alleviate the UVB-induced skin inflammation in mice.[21] In October 2021, Cao et al. in Washington University in USA found that UVB irradiation induces less scratching behavior in TPRV1 deficient mice than it does in wild type mice. Moreover, UVB irradiation on the DRG cells induces transcriptional upregulation of TRPV1 and TRPA1, along with the enhancement of intracellular calcium signals by capsaicin at 100nM or KCl at 100 m[22]. However, in these experiments, they did not address whether UV regulates the protein expression level of TRPV1 and TRPA1. Moreover, whether UVB irradiation by itself would induce intracellular calcium propagation remains unclear.

4. Study design

I irradiated human keratinocytes and mouse DRG cells with different intensities of UVB and measure the protein expression of TRPV1/TRPA1 using immunofluorescent exam and flow cytometry. I also measured and compared the dynamic calcium propagation in these cells irradiated with UVB.

5. Methods

5.1.UV irradiation

The cells were irradiated with a UVB source (SVL1, 311 nm, 1 × 15 W, intensity 1.28 mW/cm^2, France). The equipment was calibrated by a UVX digital radiometer (UVR-305/365-D detector, Tokyo Optical Co., LTD.). This calibration allowed an energy density of 1 mW/cm^2 with a height of 15 cm.

5.2.Cell incubators

The cells were harvested in the incubator with a constant CO_2 and O_2 concentration.

5.3.Optical microscopy for calcium imaging and immunofluorescence exam

The optical microscope of BX43 (Olympus) was used.

5.4.Immunofluorescent stain

For DRG cells, TRPA1 was stained with Novus NB110-40763 (1:200, 5 ug/ml, red). TRPV1 was stained with Novus NBP1-71774 (1:200, 5 μg/ml, green). DAPI (blue) was used for nuclei staining. For keratinocytes, TRPA1 was stained with Novus NB110-40763 (1: 200, 5 ug/ml, red). TRPV1 was stained with Invitrogen PA1-748(1: 200, 5 μg/ml, green), DAPI (blue) was used for nuclei staining.

5.5.Calcium imaging

Cells were washed with PBS (phosphate-based saline) to ensure no calcium in the buffer. Flou-4 (Thermo Fisher, F14201) was used for calcium imaging at 5ug/ml at 37degrees in Celsius. This calcium imaging apparatus was located in the Bioimaging Core Facility in National Cheng-Kung University in Tainan. The instrument was partly supported by Ministry of Science and Technology “. https://bioimage.med.ncku.edu.tw/upload/apply/files/2022050613301830.pdf
The target cells were chosen to measure the light intensity from the region of interest. The ration of F340/F380 was used as an indicator for the intracellular calcium signals.

5.6.Flow cytometry

After the cells were stained with corresponding antibodies against TRPV1 or TRPA1, the cells were observed and analyzed using multi-color flowcytometry (BD Bioscience) with the category of unstained, isotype, and target antibody group.

5.7.DRG cells

The primary culture of DRG cells could be based on the Perner and Sokol from Harvard University, however, the dissection of the spine is required[23]. We decided to purchase the Rat dorsal root ganglion neurons (RDRGN, Sigma-Aldrich R8820N-05) for the sake of laboratory regulations. The DRG cells were harvested with the DRG culture medium (Sigma-Aldrich and Thermo Fisher), which contains Neurobasal-A medium, Penicillin/streptomycin, B-27 supplement, GlutaMax, NGF 2.5 S (50 ng/ml), GDNF (2 ng/ml), Ara-C (10 μM).

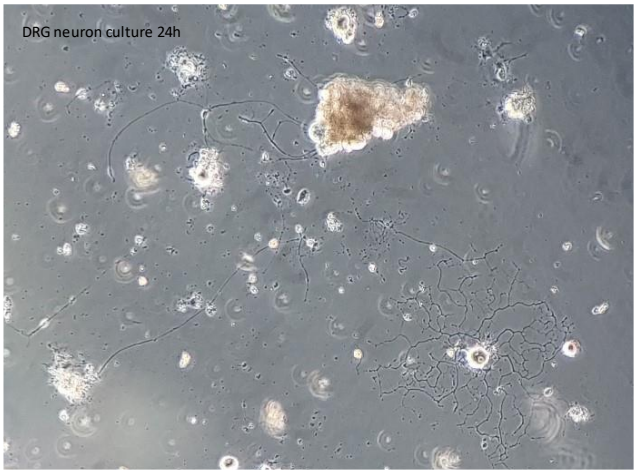
5.8.Human keratinocytes

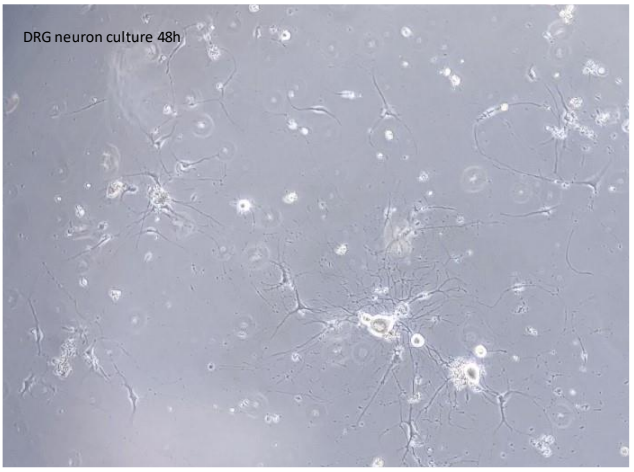
The primary human epidermal keratinocytes were purchased from ATCC (PCS-200-011) through the distributor of (distributor Union BioMed Inc. Taiwan).

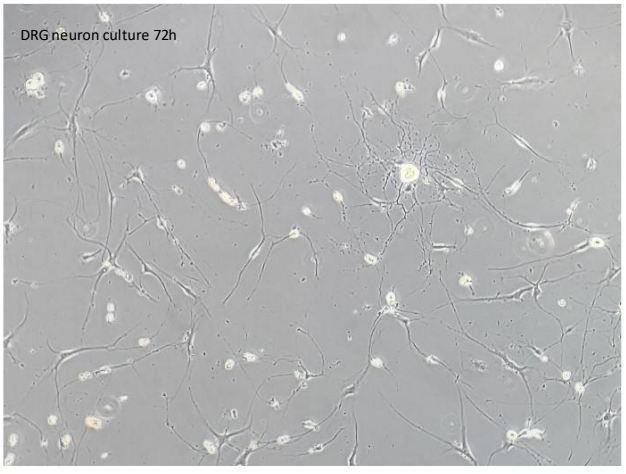
6. Results

6.1.Culture of DRG cells

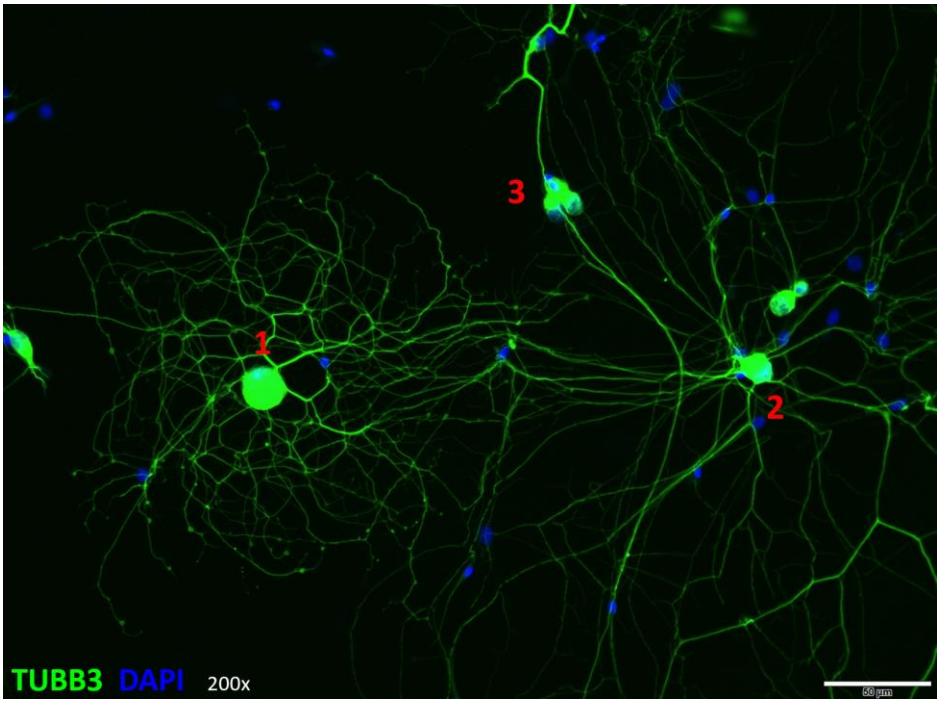
In order to see whether the morphological features of DRG cells could be observed, I harvested the DRG cells for 24, 48, and 72 hours and observed them under optical microscope. The result showed that some cells with dendritic formation were being developed. The dendritic features indicated the nerve origin of the cells.





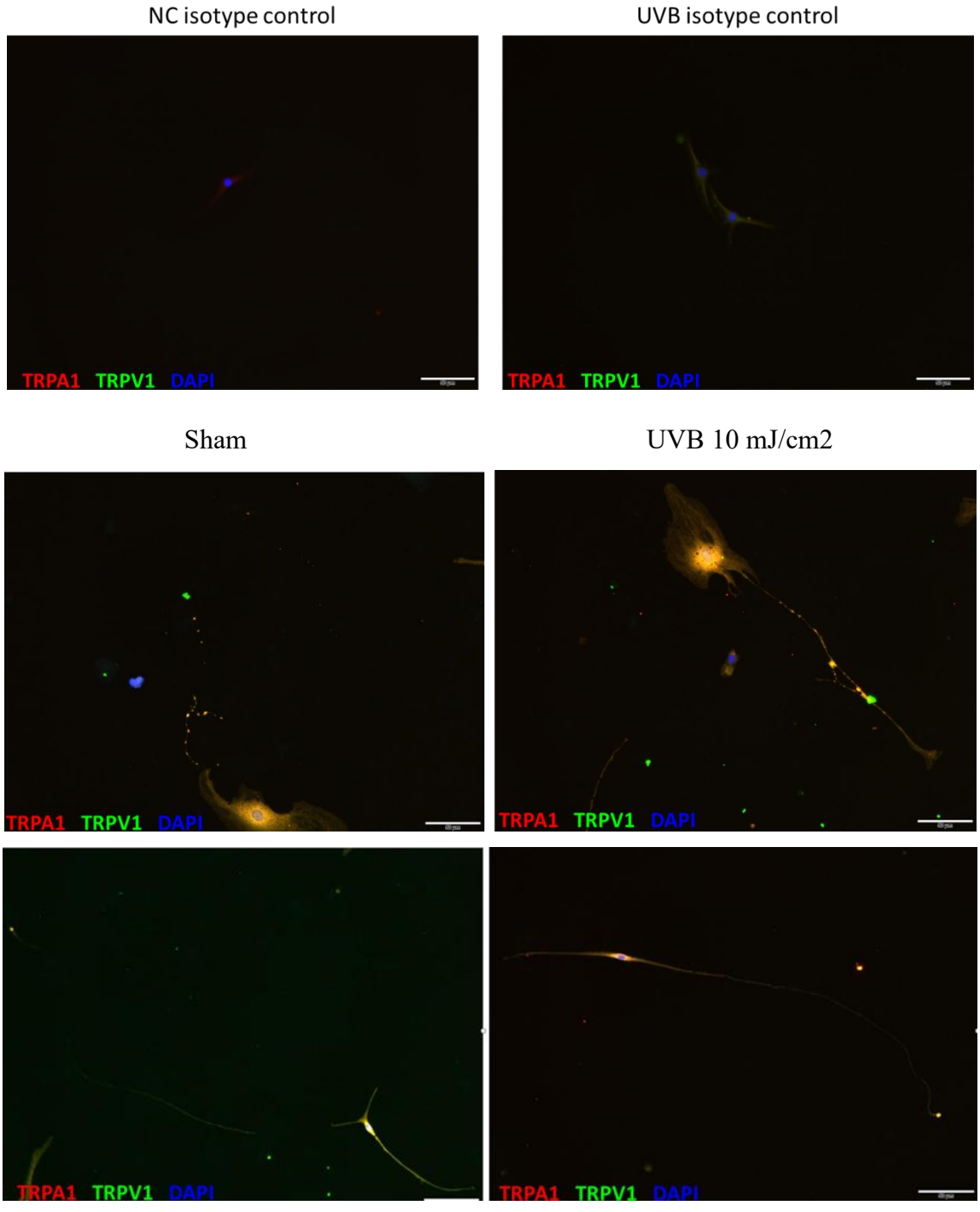


At 48 hours after irradiation, cell skeleton is highlighted by tubulin3 (TUBB3) by immunofluorescence. The results showed characteristic branches, which feature the neuron cells.



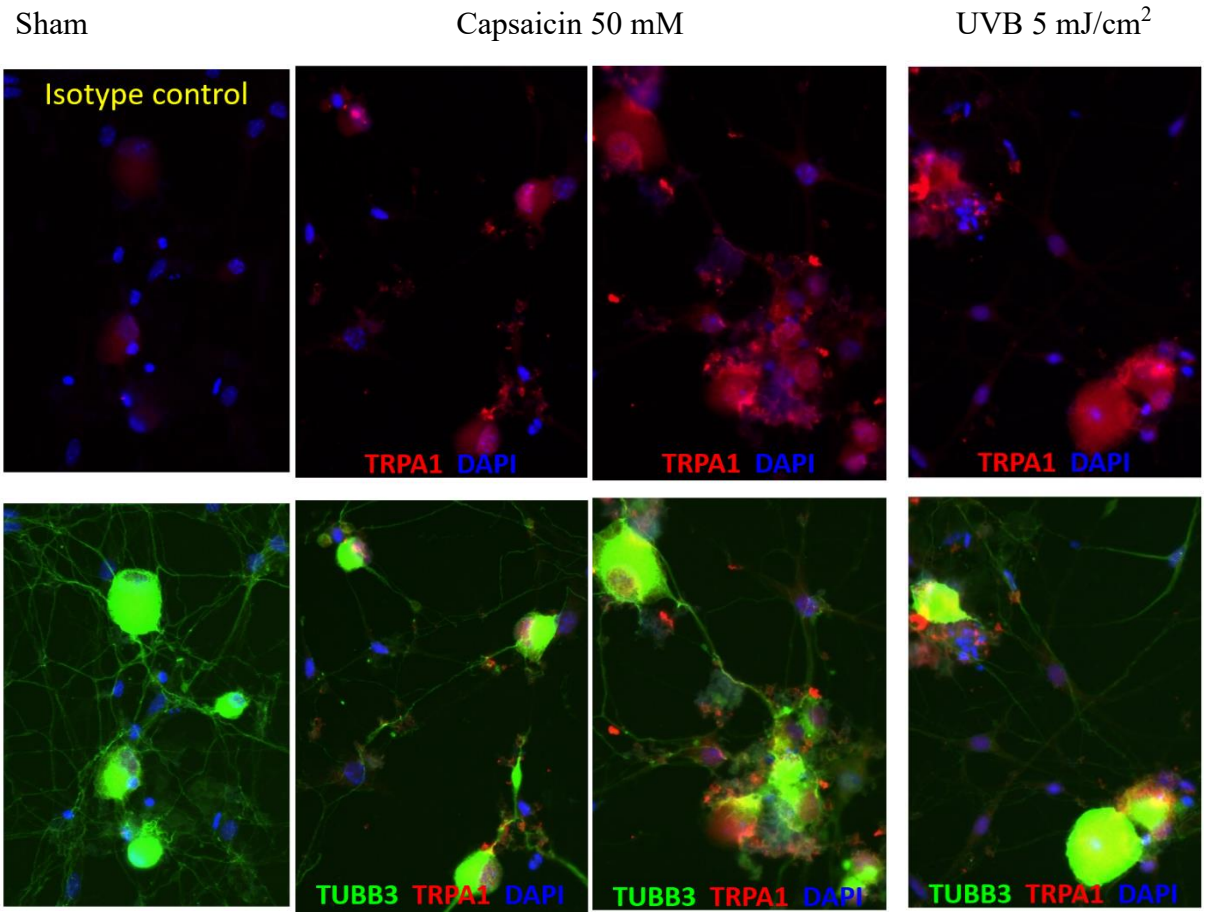
6.2.UVB at 10 mJ/cm^2 modestly enhanced the TRPA1 and TRPV1 expression in DRG cells

To investigate whether TRPA1 and TRPV1 expression in DRG cells would be regulated by UVB irradiation, DRG cells were irradiated with UVB at 10 mJ/cm^2 and stained with corresponding TRPV1/TRPA1 antibodies. DAPI was used to stain cell nuclei. TRPA1 was detected by the red conjugated antibody (Novus NB110-40763 (1:200, 5 ug/ml)) while TRPV1 was detected by green conjugated antibody (Novus NBP1-71774 (1:200, 5 ug/ml)). The results showed that before UVB irradiation, there are low expression of TRPV1 and TRPA1 in DRG cells. After UVB irradiation, the expression of TRPV1 and TRPA1 are both modestly enhanced.

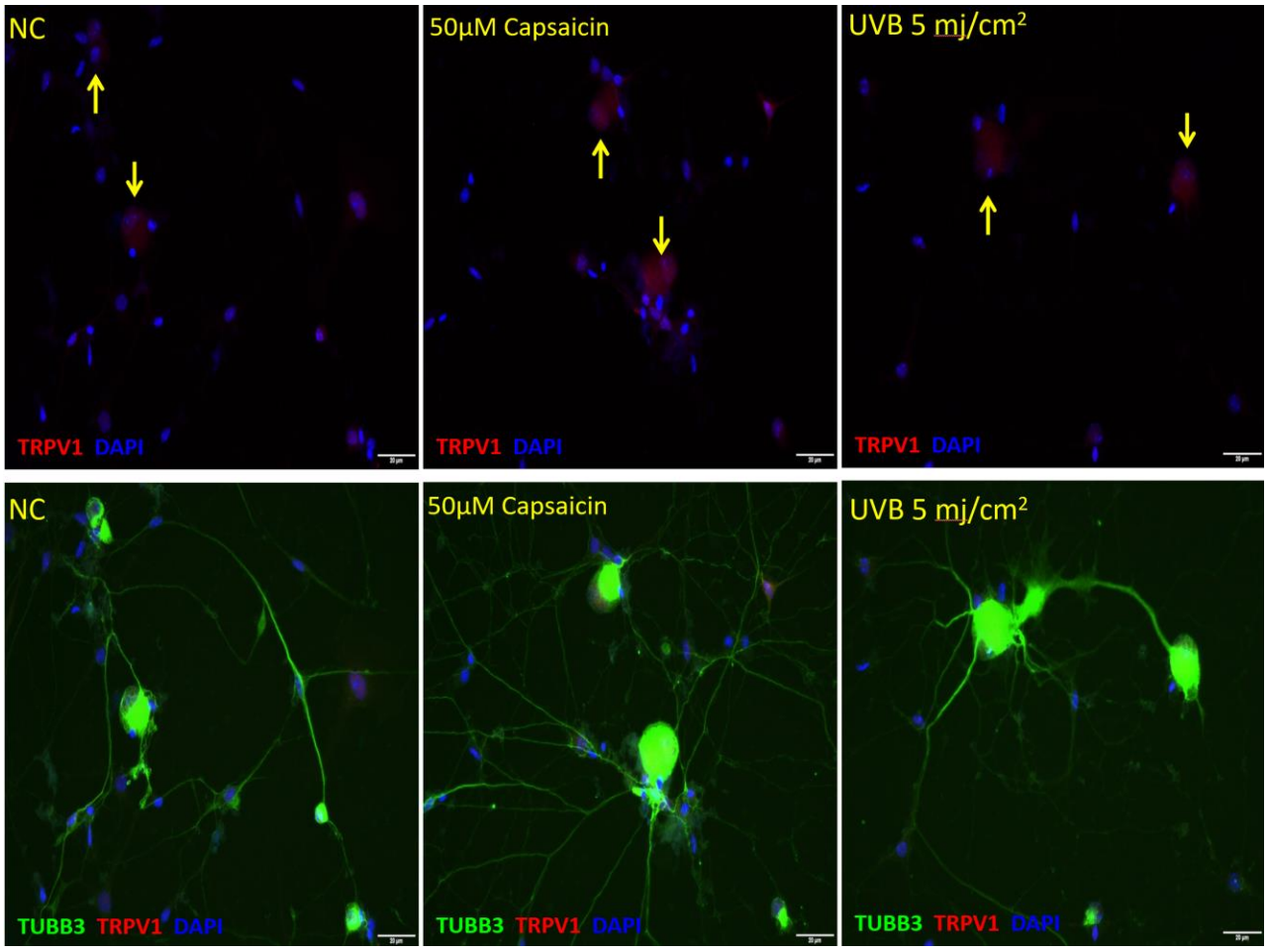


6.3.UVB irradiation at 5 mJ/cm^2 induced a significant expression of TRPA1 but not TRPV1

Next, I irradiation the DRG cells at a lower energy of UVB at 5 mJ/cm^2 and incorporated a positive control group where cells were treated with capsaicin at 50mM, to see whether lower UVB energy irradiation would affect the expression of TRPV1/TRPA1.

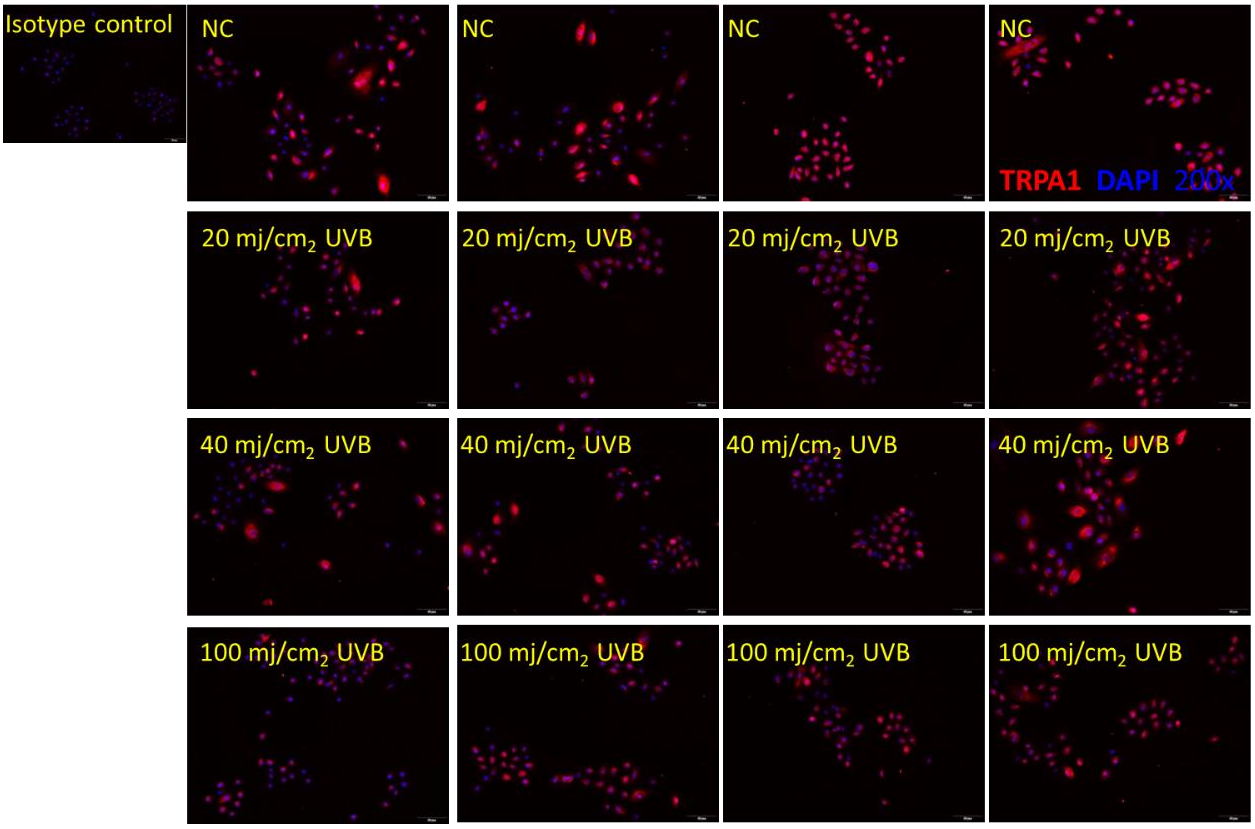


The results showed that either UVB irradiation at 5 mJ/cm^2 or capsaicin treatment could induce a significant expression of TRPA1. However, the expression of TRPV1 on DRG cells was not affected by capsaicin or UVB at 5 mJ/cm^2 (Figure below).


NC
50μM Capsaicin
UVB 5 mj/cm2
TRPV1 DAPI
TRPV1 DAPI
TRPV1 DAPI
NC
50μM Capsaicin
UVB 5 mj/cm2
TUBB3 TRPV1 DAPI
TUBB3 TRPV1 DAPI
TUBB3 TRPV1 DAPI

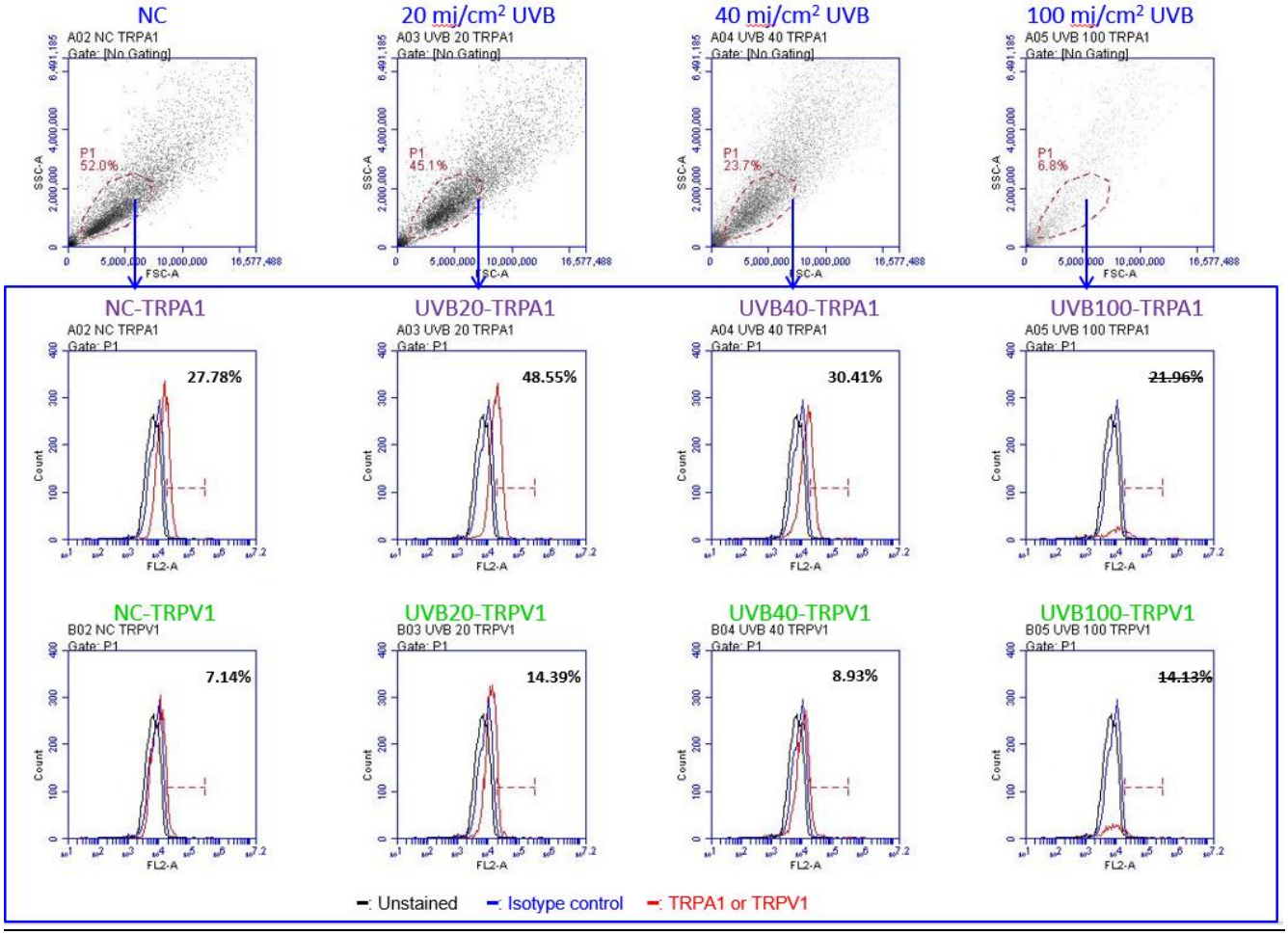
6.4.Cytotoxicity of UVB at 20 mJ/cm^2 on keratinocytes

I thought to determine the effects of UVB on cultured keratinocytes at various intensity of 20, 40, and 100 mJ/cm^2. The result showed that with such intensities, the numbers of keratinocytes are significantly decreased. In the same time, the expression of TRPA1 is not changed nor enhanced by UVB at 20, 40, and 100 mJ/cm^2. Hence, in the following experiments, I decided to use 15 mJ/cm^2 or lower as the irradiation intensity for keratinocytes (TRPA1, Novus NB110-40763；TPRV1 Invitrogen PA1-748).

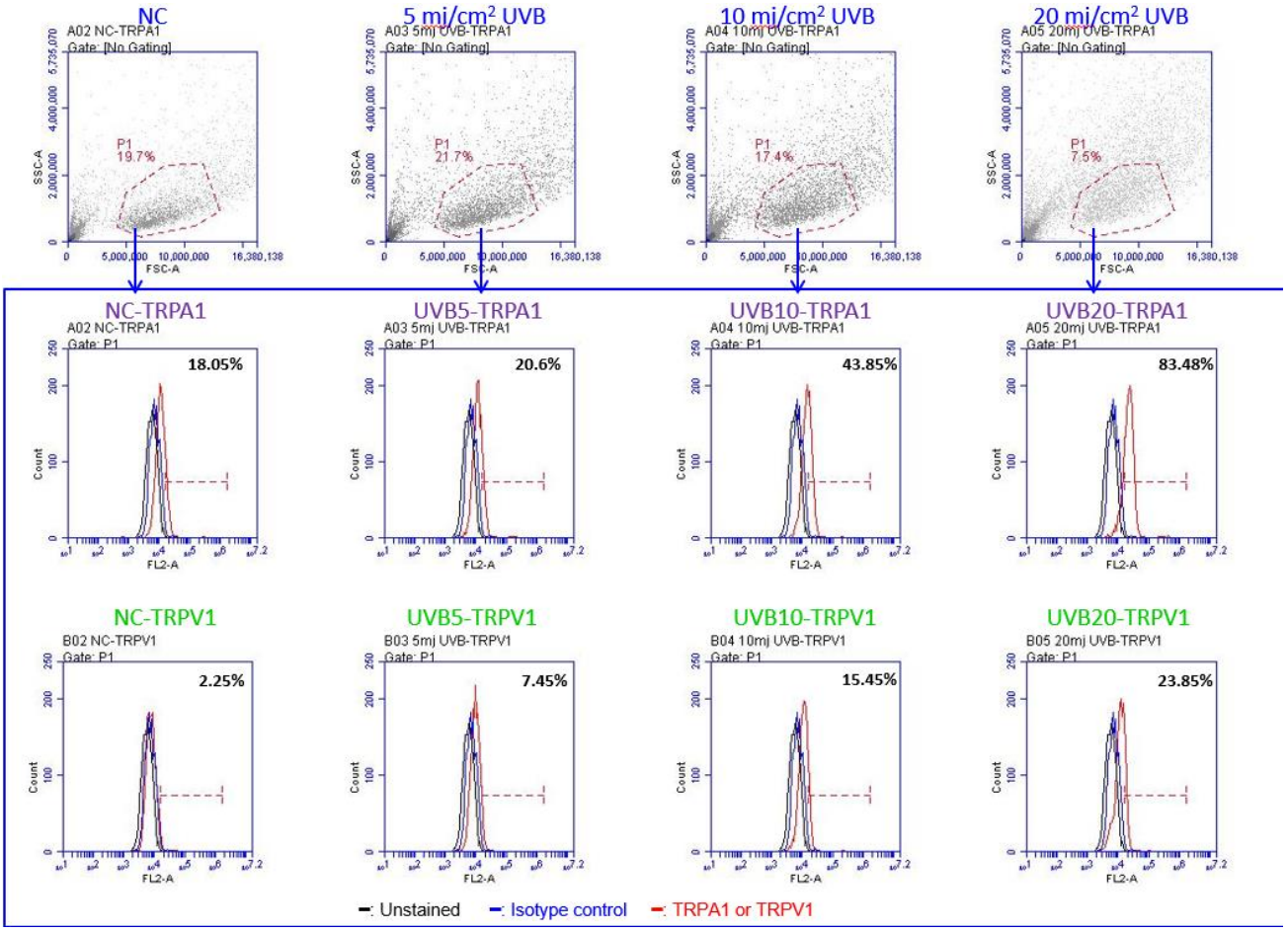


6.5.UVB irradiation at 10 mJ/cm^2 induced a significant expression of both TRPV1 and TRPA1 in keratinocytes by flow cytometry

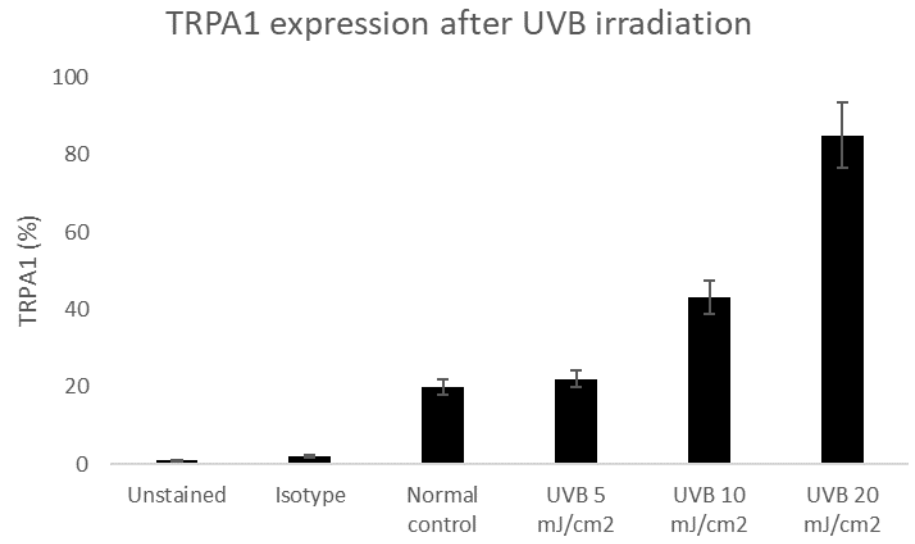
When keratinocytes are irradiated with UVB at 100 mJ/cm^2, the numbers of keratinocytes are robustly decreased, indicating a strong cytotoxicity at this intensity. I irradiated the cells with UVB at the lower intensity at 20 and 40 mJ/cm^2. The results showed that while UVB irradiation at 40 mJ/cm^2 did not affect the expression of TRPV1 and TRPA1 significantly, UVB irradiation at 20 mJ/cm^2 induced TRPA1 expression from 27% to 48% and TRAP1 expression from 7% to 14%. The results indicated that UVB irradiation at 20 mJ/cm^2 induced a significant expression of both TRPV1 and TRPA1 in keratinocytes.

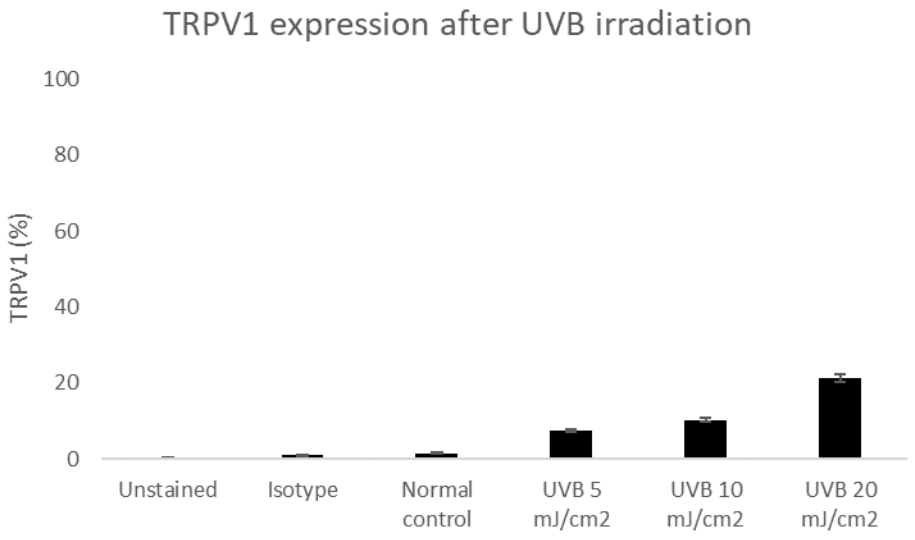


Since TRPV1/TRPA1 expression in cells irradiated with UVB at 40 mJ/cm^2 were decreased than those irradiated with UVB at 20 mJ/cm^2. I then asked whether lower intensity of UVB would do the same thing. I irradiated the keratinocytes with nbUVB at 5, 10, or 20 mJ/cm^2 for 24 hours and measured the expression of TRPV1 and TRPA1 expressions in keratinocytes. The results showed although UVB at 20 mJ/cm^2 induced a significant expression of TRPA1(18%->83%) and TRPV1(2%->24%), it reduced the cell numbers robustly, indicating a cytotoxicity of UVB at 20 mJ/cm^2. UVB at 10mJ/cm^2 induced an even higher expression of TRPA1(18%->43%) and TRPV1(2%->15%) than it did at 5mJ/cm^2. In summary, I found UVB irradiation at 10 mJ/cm^2 induced significant expression of both TRPV1 and TRPA1 in keratinocytes.



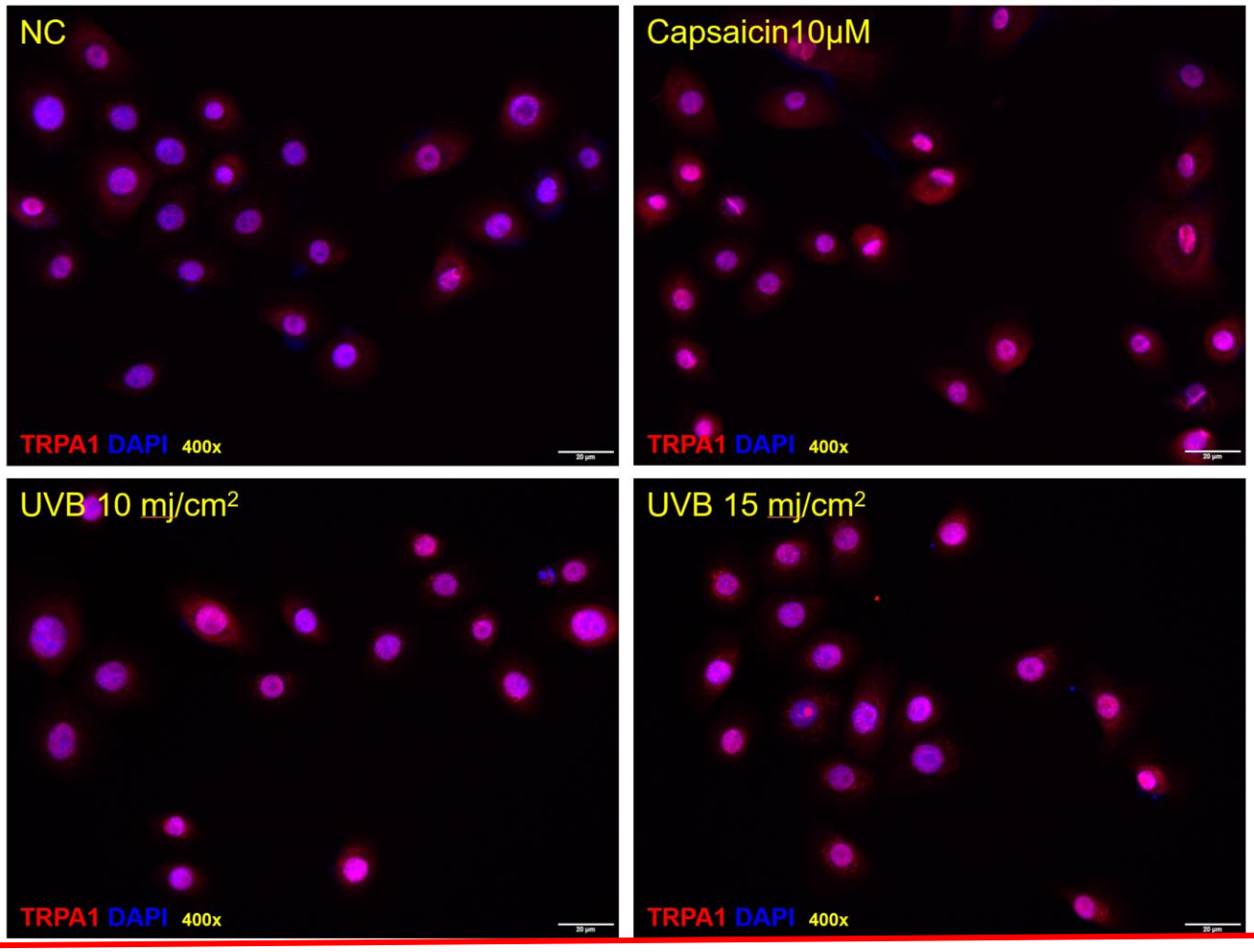
I quantified the results of these two experiments, showing the significant expression of TRPA1 and TRPV1 in keratinocytes 24 hours after irradiated with UVB at either 10 or 20 mJ/cm^2.

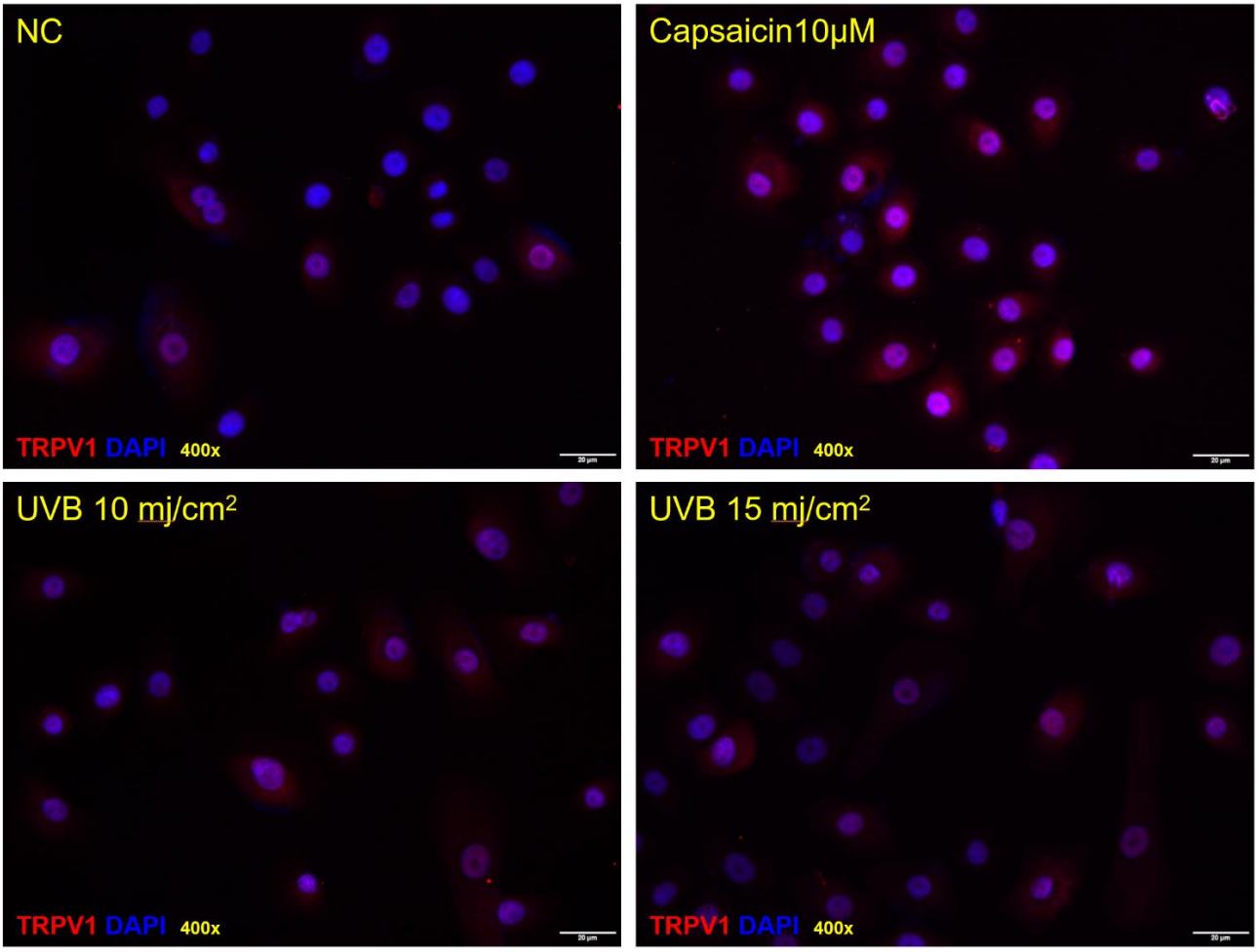




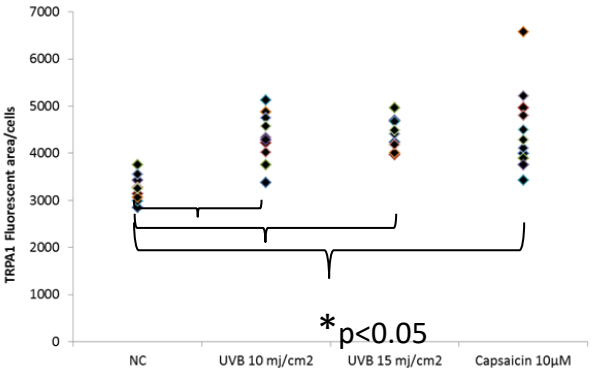
6.6.UVB irradiation induced a significant expression of both TRPV1 and TRPA1 in keratinocytes by immunofluorescence

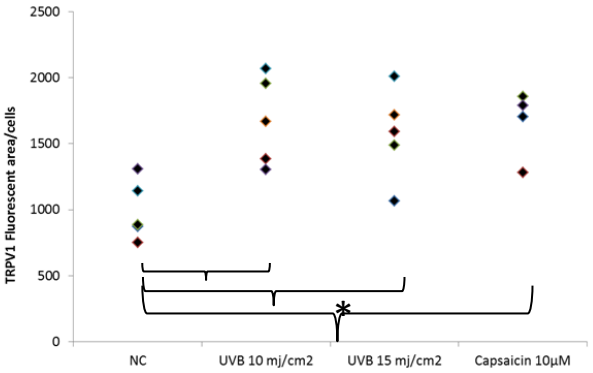
Since I found UVB irradiation at 10 mJ/cm^2 induced TRPV1/TRPA1 expression in keratinocytes, while UVB irradiation at 20 mJ/cm^2 induced cell toxicity by flow cytometry, I then decided to irradiate the keratinocytes at 10-15 mJ/cm^2 UVB and see whether the expression of TRPV1 and TRPA1 would be consistently increased by immunofluorescent exam. The results showed the positive control using capsaicin induced significant expression of both TRPV1 and TRPA1.





The results showed that UVB at both 10 and 15 mJ/cm^2, induced a significant expression of both TRPV1 and TRPA1. Of note, the induction of TRPA1 was higher than that of TRPV1. The results obtained by immunofluorescent recapitulate the findings measured by flow cytometry.

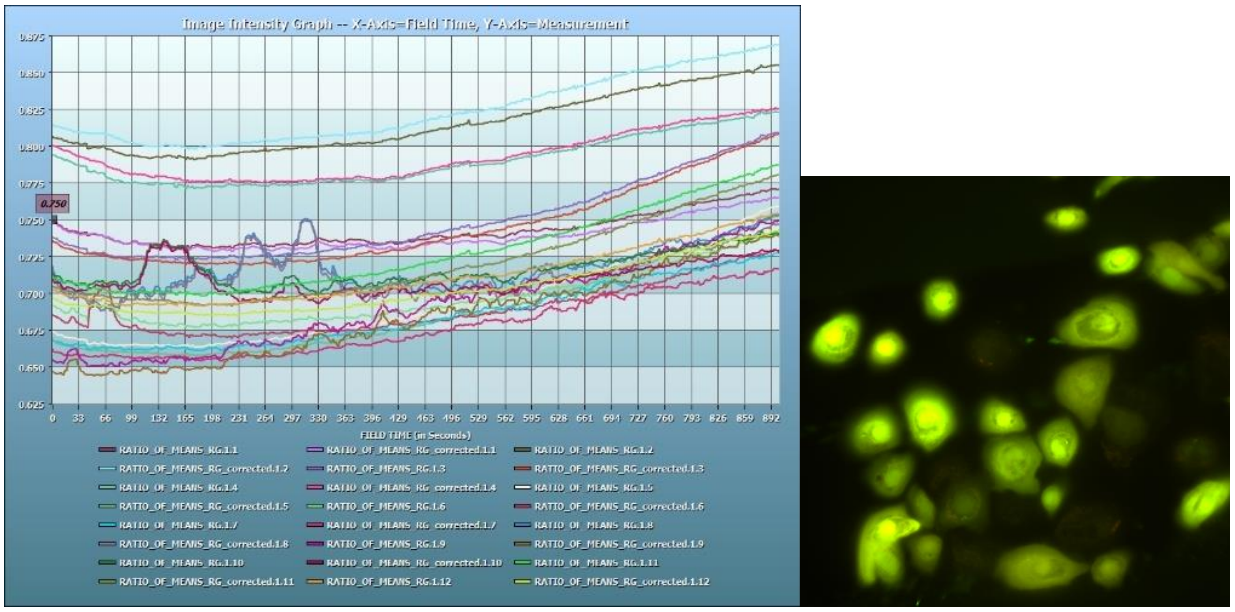




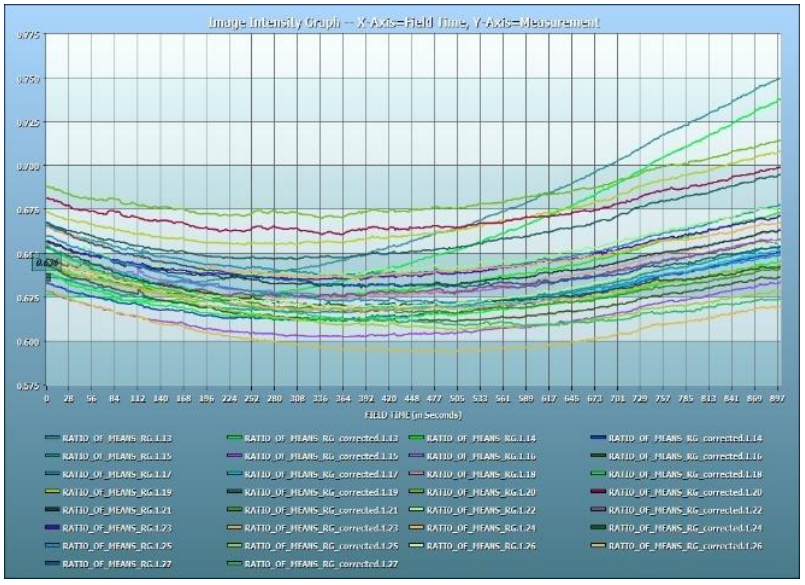
6.7.No obvious changes of dynamic calcium imaging in keratinocytes irradiated with UVB

I saw an increased expression of TPRV1 and TRPA1 after UVB irradiation. I then asked whether the functional changes could be seen after the TRPV1 and TRPA1 activation. To do so, I measured the dynamic calcium imaging in keratinocytes preincubated with Flou 4 and treated with or without UVB. The results showed no significant changes of dynamic calcium imaging in keratinocytes irradiated with UVB at 15 mJ/cm^2.

Sham



UVB 15 mJ/cm^2



Since the calcium imaging equipment did not include other photon lasers, we could not measure the dynamic expression of UVB on the calcium immediately after UVB irradiation. Alternatively, I think cell calcium could be activated by Thapsigaragin or ATP, and then irradiated with UVB to follow the calcium response after UVB.

7. Discussion

In this study, I showed that UVB induced a modest PROTEIN expression of TRPA1 and TRPV1 in DRG cells. For keratinocytes, UVB irradiation induced a dose-dependent increase of TRPA1 and TRPV1 protein expression by flow cytometry. The calcium propagation in keratinocytes seems similar when they are irradiated with UVB.

The study first found that UVB induced a modest protein expression of TRPA1 and TRPV1 in DRG cells. The time dynamic measurement and dose titration may be tried to see whether more differences of TRPA1 and TRPV1 expression could be observed. The other way around will be made possible by using TRPV1-GFP transgenic mouse to directly measure the TRPV1 expression without the immunostaining (GENSAT program, MMRRC services; UC Davis, CA).

In my experiments when keratinocytes were irradiated with UVB, I measured the calcium propagation after the UVB irradiation and I failed to observe the meaningful changes of the dynamic calcium signals with or without UVB irradiation. I am now trying to use a build-in UVB source incorporated into the confocal microscope for calcium imaging so that the real time dynamics of calcium signals could be observed IMMEIDATELY after the UVB irradiation. The irradiation on one cell or a group of cells nearby could be applied to measure the time dynamics of calcium signals.

To date, only three articles have investigated the effect of UVB on the expression of TRPV1/TRPA1 in DRG cells. First, Cao et al. found that UVB irradiation induced less scratching behavior in TRPV1-deficient mice; They found UVB induced increased transcriptional level of TRPV1 and TRPA1 and potentiated the calcium response induced by capsaicin 100nM and KCl 100 mM[22]. However, in their study, they did not measure the protein level of TRPV1 and TRPA1 after UVB irradiation. The rest of two publications mainly investigated how UVB induced TRPV1 and TRPA1 expression INDIRECTLY thought the inflammation that UVB elicited[21, 24]. Therefore, my results are significant with scientific advances. What I have been investigating is the protein level of TRPV1 and TRPA1 but not the transcriptional level. Moreover, I studied the DIRECT effects of UVB on TRPV1 and TRPA1, which is independent of the UVB-induced inflammation. In the future, I will measure the time dynamic calcium signals after UVB irradiation. More functional results could be obtained.

The intracellular signals after UVB activation or TRP activation could be measured later on. More sophisticated experiments with more consistency and dose titration might be warranted. Nevertheless, with the short time of several months in the high school, I managed to familiar with the dynamic calcium imaging. The microscopical field of individual cells after calcium propagation is just like the admiral twinkling firefly lights in the night.

8. Conclusion

In this study, I showed that UVB induced a modest protein expression of TRPA1 and TRPV1 in DRG cells. For keratinocytes, UVB irradiation induced a dose-dependent increase of TRPA1 and TRPV1 protein expression by flow cytometry. The calcium propagation in keratinocytes seems similar when they are irradiated with UVB. The induction of TRPA1 and TRPA1 in both keratinocytes and DRG cells might be related to the warmth sensation after sunlight irradiation.

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【評語】090011

研究主題有創意， 分析與顯微鏡照片相當細緻，已具專業的規模。實驗架構可能需要再修改，例如為何 DRG 在體內但需要做 UVA/B 的照射？Keratinocyte 有多少的 TRPV1/A 的表現量，和其他離子通道比起來，這樣的表現量是的確具有生理功能嗎？