약학석사학위논문

# 촉각관련세포에서의 기계채널 전류

## Mechanosensitive Currents in Merkel Cells

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# ABSTRACT

Merkel cells are mechanosensory cells known to be ‘touch cells’ about 160 years ago. However, its mechanism of how transduce and encode for touch sensation is still unknown. The objective of the present study is to characterize mechanosensitive currents in Merkel cells and investigate which ion channels are related to mechanotransduction in Merkel cells.

By patch-clamp technique, we characterized mechanically activated (MA) currents in cultured mouse Merkel cells from whisker hair follicles. Merkel cells displayed mixed MA currents, rapidly adapting and slowly adapting currents. We confirmed that those currents were MA currents since they were inhibited by Gadolinium ($Gd^{3+}$) and GsMTx4. Piezo2 and Tentonin3 ion channels were highly expressed in cultured mouse Merkel cells. We investigated the function of Piezo2 and Tentonin3 in Merkel cells. Knockdown of Piezo2 and Tentonin3 by siRNA transfection significantly reduced amplitude of MA currents in Merkel cells. Interestingly, Tentonin3 siRNA transfected Merkel cells showed higher reduction percentage of slowly adapting currents than other current types.

We proposed that Tentonin3 plays role in MA currents in Merkel cells, especially in slowly adapting currents. This study on mechanosensitive currents in Merkel cells will provide basic information of Tentonin3 and more research remains to be done to understand function of Tentonin3.

Keywords: Merkel cell, Piezo2, Tentonin3, Touch, Mechanosensitive channels

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# INTRODUCTION

We rely on our sense of touch to gather information about the world around us. Without touching sensation we will not be able to survive. Mechanotransduction, the conversion of mechanical force into biological signals has crucial roles in our life. Touching, pain, hearing, and cell volume regulations are all regulated by mechanostransduction. However, only few mechanically activated channels have been identified to date. And how we sense touch remains fundamentally unclear.

The Merkel cell is a gentle touch receptor found in the skin. It is abundant in fingertips of humans and whisker hair follicles of nonhuman mammals. It is known that Merkel cells form synapse-like structure with nerve endings, Merkel cell-neurite complex, and transduce light touch or hair movements into mechanical stimulation. Although, Merkel cells were discovered about 160 years ago by Friedrich Sigmund Merkel, cellular and molecular mechanism underlying mechanotransduction in Merkel cells are still debating.

Recent studies revealed that Merkel cells rapidly transduce mechanical stimuli through Piezo2 ion channel (Coste et al., 2010). However, how Merkel cells transduce and encode touch sensation remain unknown. Only few mechanically activated channels have been identified. In this regards, we searched for new transmembrane protein ‘Tentonin3’ as possible a regulator of mechanotransduction.

The purpose of the present study was to establish culture condition of Merkel cells and to characterize mechanosensitive currents in Merkel cells and finally to investigate which ion channels are related to mechanotransduction in Merkel cells.

# MATERIALS AND METHODS

## 1. Mouse Merkel Cell Culture

C57/BL6 mice aged 1-4 days were sacrificed by decapitation. Whisker pads were removed and placed in a dish filled with ice cold Dulbecco's Modified Eagle's medium (DMEM, Gibco). Under a light microscope, individual whisker hair follicles were dissected out from whisker pads of mice. Free follicles were transferred to a tube containing 6 mg of collagenase type II (Worthington) in 2 ml of DMEM and incubated at 37℃ for 30 minutes on shaker water bath. Enzyme activity was ceased by washing follicles three times with DMEM. Merkel cells were dissociated from the follicles by repeated trituration with a Pasteur pipette. The dissociated cells were plated on glass coverslips pre-coated with poly-L-lysine (Sigma) and maintained in a DMEM with 5% heat-inactivated fetal bovine serum (FBS, Gibco) and 1% penicillin (Gibco). The coverslips were incubated for 30 minutes in culture medium at 37℃ in a $CO_2$ incubator. After 30 minutes, cells that failed to attach were removed by washing. The coverslips with cells attached were incubated in the growth medium in 35 mm plastic dishes. The cultures were maintained at 37℃ in a humidified atmosphere of 95% air and 5% $CO_2$ for 24 hours.

## 2. Total RNA Purification and cDNA Synthesis

Total RNAs used for RT-PCR were purified from cultured mouse Merkel cells using easy-spin™ Total RNA Extraction Kit (iNtRON Biotech, Korea) according to the manufacture's protocol. Concentration of purified total RNA was measured by Nanodrop (Thermo). cDNAs were synthesized using the cDNA Synthesis Kit (Promega Corporation).

## 3. Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Using cDNAs synthesized from mouse Merkel cells, reverse transcription polymerase chain reaction (RT-PCR) was performed using the DreamTaq DNA Polymerase (Thermo Scientific™). Amplification conditions were 3 minutes of initial denaturation at 95℃, followed by 35 cycles of each 30 seconds at 95℃, 30 seconds at 58℃, 40 seconds at 72℃, and final extension at 72℃ for 10 minutes. Primer sequences used are listed in Table1.

PCR products were resolved by electrophoresis in 1% agarose gels, stained with ethidium bromide and visualized under U.V. light.

## 4. Small Interfering RNA Transfection

Small Interfering RNA (siRNA) targeting mouse Piezo2, Tentonin3 or scrambled siRNA were transfected into cultured Merkel cells using Lipofectamine 2000™ (Invitrogen) 1day after plating. After 48 hours of transfection, whole-cell patch-clamp recordings from siRNA treated Merkel cells were performed. Target sequences for siRNA are listed in Table2.

## 5. Immunocytochemistry

To stain rat whisker hair follicles with Quinacrine, individual whisker hair follicle was incubated with 300 nM Quinacrine in Hank's balanced salt solution (HBSS) for 15 minutes at room temperature. To stain cultured Merkel cells with Quinacrine, a round glass coverslip with cultured Merkel cells were incubated for 1 hour with 100 nM Qunacrine in HBSS at 37℃.

For immunostaining, Merkel cells cultured on glass coverslips were fixed with a 4% paraformaldehyde (PFA) for 15 minutes. 0.1% Triton X-100 in PBS was used for 10 minutes at room temperature for permeabilization. Cultured Merkel cells were labelled at 4℃ overnight with primary antibodies against Cytokeratin 20 (mouse monoclonal antibody, Abcam), Piezo2 (rabbit polyclonal antibody, Atlas) and Tentonin3 (rabbit polyclonal antibody).

Primary antibodies were used at 1:100 dilution in 1% Albumin for Bovine serum (BSA) in phosphate buffered saline (PBS). Secondary antibodies (Alexa Fluor® 488 Donkey Anti-Mouse IgG, Alexa Fluor® 546 Goat Anti-Rabbit IgG) were loaded with 1:200 dilution in 1% BSA solution for 1 hour at room temperature. After being washed with PBS, Hoechst 33342 (Molecular Probes®) were loaded with 1:500 dilution in PBS for 5 minutes at room temperature. Coverslips were mounted on glass microscope slides with permanent aqueous mounting medium (Biomeda, Foster City, CA) and waited 30 minutes to dry.

## 6. Electrophysiology

Currents were recorded from Merkel cells after 1-2 days in culture with Axopatch 200B (Axon Instruments, Molecular Devices Inc.). Pipettes were pulled from borosilicate glass capillaries using a P-97 micropipette puller (Sutter Instruments Co.) and fire polished by a MF 820 microforge (Narishige, Japan) to have resistance of 2-4 megaohms when filled with the pipette solution. Currents in the whole-cell configuration were recorded in extracellular solution containing (in mM): 5 KCl, 140 NaCl, 2 $MgCl_2$, 2 $CaCl_2$ and 10 HEPES (pH7.2, adjusted with NaOH). The pipette solution contained (in mM): 140 KCl, 2 $MgCl_2$, 5 EGTA, 2 MgATP, 0.3 NaGTP and 10 HEPES (pH 7.2, adjusted with NaOH). To determine membrane action potential properties of Merkel cells, under whole-cell configuration, step current pulses were injected into cells

through patch-clamp electrodes from -80 pA to 200 pA in increments of 40 pA per step and the duration of each pulse was 200 ms.

## 7. Mechanical Stimulation

To determine whole-cell currents evoked by mechanical stimulation (MA currents), Merkel cells were stimulated for 600 ms by 0.21 μm displacement steps with a glass probe (tip diameter: 2-3 μm) driven by a piezoelectric actuator (Kleindiek Nanotechnik). The glass probe was positioned near cell surface at an angle of 55°. Merkel cells were voltage-clamped at -60 mV when mechanically evoked currents were measured.

## 8. Pharmacological Test

For the Gadolinium (Sigma) blockade experiment, 100 mM stock solution was dissolved into extracellular solution at a final concentration of 100 μM.

For GsMTx4 blockade experiment, 25 μM stock solution was dissolved into extracellular solution at a final concentration of 2.5 μM.

Mechanically activated currents in Merkel cells by glass probe displacement stimulation were tested in normal extracellular solution as controls, then extracellular solution contained Gadolinium or GsMTx4 were applied for

3 minutes. MA currents were recorded again at least three times. After wash out with normal extracellular solution, MA currents were recorded again.

## 9. Statistical Analysis

All data are expressed as mean± SE of at least three independent experiments were calculated. Data analysis was performed by Clampfit 10.2 software or SigmaPlot 10.0 software. Statistical differences between groups were assessed using unpaired t-test, whereby * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ were considered as statistical significance.

| Gene | F/R | Sequence (5'→3') | Size |
|---|---|---|---|
| Mouse GAPDH | F | AGGTCGGTGTGAACGGATTTG | 400 bp |
| | R | TGTAGACCATGTAGTTGAGGTCA | |
| Mouse Piezo1 | F | ACTGGCTGCTGATGACCTCT | 415 bp |
| | R | TTCAGGAGAGAGGTGGCTGT | |
| Mouse Piezo2 | F | ACCGATCGTGGAAGTAGTGG | 417 bp |
| | R | AGGTGCTGGGTAATCTGGTG | |

**Table1. Primers for RT-PCR**

| Gene | Sense/ Antisence | Sequence (5'→3') |
|---|---|---|
| Mouse Scramble siRNA | Sense | CCUACGCCACCAAUUUCGU |
| | Antisence | ACGAAAUUGGUGGCGUAGG |
| Mouse Piezo2 siRNA | Sense | GAAUGUAAUUGGACAGCGA |
| | Antisense | UCGCUGUCCAAUUACAUUC |

**Table2. Small Interfering RNA (siRNA) Sequences**

# RESULTS

## 1. Identification of Merkel cells using Quinacrine staining

Merkel cells in rat whisker hair follicle were identified by staining with Quinacrine, a fluorescent marker for Merkel cells. Under the fluorescent microscope, we could locate Merkel cells in whisker hair follicles. Compare to intact whisker hair follicle (Fig.1A), stained Merkel cells in whisker hair follicle showed green fluorescent (Fig.1B). Also in dissociated cultured Merkel cells from whisker hair follicles, we could see fluorescent of Quinacrine stained Merkel cells (Fig.1C). Merkel cells were round in shape and they had some fibroblast like processes in culture overnight.

## 2. Piezo2 and Tentonin3 mRNA expression in mouse Merkel cells

The mRNA expression of Piezo2 and Tentonin3 was confirmed by RT-PCR in mouse Merkel cell culture from whisker hair follicles. As a result, Piezo2 and Tentonin3 was highly detected, implying the possibility of its role in touch sensation (Fig.2)

## 3. Immunocytochemistry of cultured mouse Merkel cells

In order to confirm that Merkel cells have Piezo2 and Tentonin3, we performed immunostaining analyses of cultured Merkel cells using Cytokeratin 20 (Green) which is a Merkel cell marker. When we co-stained using Cytokeratin 20 with Piezo2 (Red) or Cytokeratin 20 with Tentonin3 (Red) antibodies, patterns were overlapped (Fig.3A and 3B).

## 4. Electrophysiological studies of mouse Merkel cells

### 4.1. Membrane action potential properties of Merkel cells

When we injected step current pulses into Merkel cells through patch-clamp electrodes under the whole-cell configuration, we found Merkel cells fired multiple action potentials in response to depolarizing current steps (Fig.4A, n = 39). This means Merkel cells are excitable cells. The Voltage-Current relationship of Merkel cells was strongly rectifying and showed a steep current-potential relationship (Fig.4B). In contrast, non-Merkel cells in whisker hair follicles never fired action potentials (Fig.5A, n = 23). And the Voltage-Current relationship was nearly linear (Fig.5B)

## 4.2. Characteristics of mechanically activated currents in Merkel cells

To see if Merkel cells display touch-activated currents, we applied mechanical force to the Merkel cell surface with piezo-driven glass probe. Under whole-cell patch clamp mode, we used another glass probe to give mechanical stimulation, gentle pocking, on the surface of Merkel cells (Fig.6A). There were two types of mechanically activated (MA) currents presented by shape and different τ-inactivation values. If the τ-inactivation value is less than 10 ms it is known to be MA currents by Piezo2 ion channels. First type of MA currents from Merkel cells were τ-inactivation < 10 ms which could be MA currents affected by Piezo2 ion channels. Second type of MA currents from Merkel cells has much bigger τ-inactivation value which indicates there are some other types of channels involved in these currents. We assumed Tentonin3 might has a major role in theses currents. We divided recorded MA currents according to their inactivation time, if their τ-inactivation time is < 10 ms we called it rapidly adapting (RA, Fig.6C), and if it is more than 30 ms, and we called it slowly adapting currents (SA, Fig.6D) and nonresponsive cells.

Mechanically activated currents in Merkel cells were displacement dependent. As we increase the displacement of mechanical stimulation, the peak amplitude of current were also increased (Fig.6B, n = 31).

## 4.3. Suppression of MA currents in Merkel cells by Piezo2 siRNA

We used siRNA transfection to examine the role of Piezo2 in MA currents of Merkel cells. Under whole-cell patch clamp configuration, we gave mechanical stimulation through glass probe on the surface of Piezo2 siRNA transfected Merkel cells. Average maximal amplitude of MA currents in Piezo2 siRNA transfected Merkel cells (9.9 ± 5.6 pA, n = 17) were significantly decreased compared to scrambled siRNA transfected cells (Fig.7B, 231.4 ± 27.1 pA, n = 38). 65% of scrambled siRNA transfected Merkel cells had τ-inactivation < 10 ms compared with 12% in Piezo2 siRNA transfected cells. Nonresponsive cells were dramatically increased in Piezo2 siRNA transfected Merkel cells, 5% in scrambled siRNA transfected cells and 82% in Piezo2 siRNA transfected cells (Fig.7C).

## 4.4. Suppression of MA currents in Merkel cells by Tentonin3 siRNA

Average maximal amplitude of MA currents in Tentonin3 siRNA transfected Merkel cells (55.2 ± 9.9 pA, n = 48) were significantly decreased compared to scrambled siRNA transfected cells (Fig.8A, 231.4 ± 27.1 pA, n = 38). Same as Piezo2 siRNA transfected Merkel cells,

nonresponsive cells were also increased in Tentonin3 siRNA transfected Merkel cells, 5% in scrambled siRNA transfected cells and 37% in Tentonin3 siRNA transfected cells. Decreased percentage of rapidly adapting MA currents was not as much as Piezo2 siRNA transfected cells. However, there were dramatic decrease in slowly adapting MA currents in Tentonin3 siRNA transfected Merkel cells. 30% of scrambled siRNA transfected Merkel cells had τ-inactivation > 30 ms compared with 17% in Tentonin3 siRNA transfected cells. These results indicating that Tentonin3 is possibly mediating slowly adapting mechanical currents (Fig.8B). Overall displacement dependently, we could see MA currents was decreased in Tentonin3 siRNA transfected Merkel cells (Fig.8C).

# 5. Pharmacology of mechanically activated currents in Merkel cells

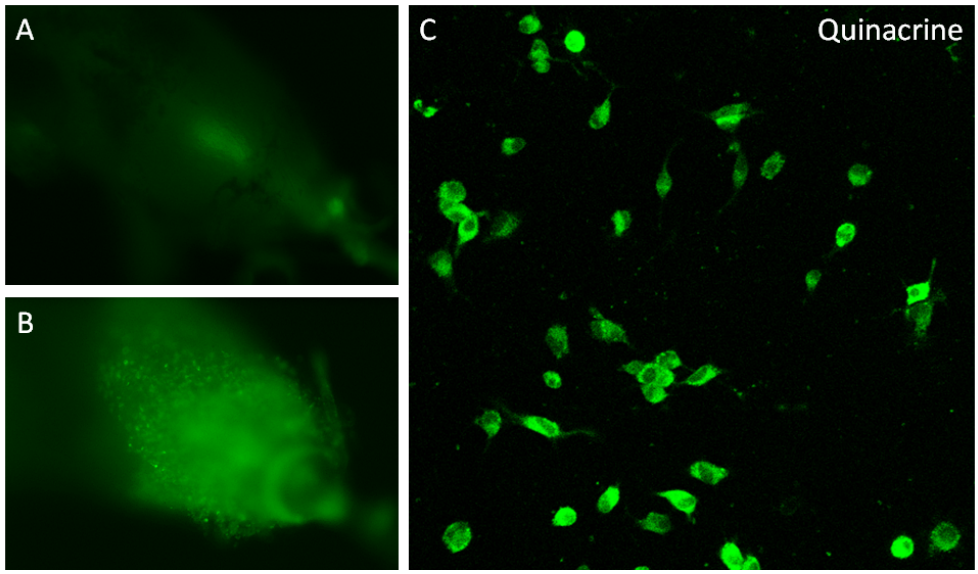
## 5.1. The effect of Gadolinium in Merkel cells

Next, we conducted pharmacological tests on MA currents in Merkel cells. Gadolinium is a well-known mechanosensitive ion channel blocker. MA currents in Merkel cells were inhibited by Gadolinium. Under whole-cell patch clamp configuration, we perfused 100 μM Gadolinium in the bath

solution and 3 minutes after, we gave mechanical stimulation on the surface of Merkel cells by 2 μm displacement. MA currents were significantly reduced by Gadolinium perfusion (Fig.9). Average maximal amplitude of MA currents were decreased from 125.9 ± 17.9 pA to 6.7 ± 4.6 pA (n = 7). However, the inhibition by Gadolinium was abolished after wash out, average maximal amplitude of MA currents, 178.5 ± 109.9 pA.

## 5.2. The effect of GsMTx4 in Merkel cells

We also tested GsMTx4, which is also a well-known mechanosensitive ion channel blocker. GsMTx4 is one of the peptide toxins isolated from the venom of the Tarantula spider. MA currents in Merkel cells were inhibited by GsMTx4 (Fig.10). Under whole-cell patch clamp configuration, we perfused 2.5 μM GsMTx4 in the bath solution and 3 minutes after, we gave mechanical stimulation on the surface of Merkel cells by 2 μm displacement. MA currents were significantly reduced by GsMTx4. Average maximal amplitude of MA currents were decreased from 263.2 ± 69 pA to 63.3 ± 12.2 pA (n = 3). However, MA currents in Merkel cells were recovered after wash off the GsMTx4, with average maximal amplitude of MA currents, 219.8 ± 22.3 pA. These data confirm that currents that we saw from Merkel cells are mechanically activated currents.




## Figure 1. Quinacrine staining of Merkel cells

A. Intact rat whisker hair follicle under fluorescent microscope.

B. Quinacrine stained rat whisker hair follicle.

C. Quinacrine stained cultured rat Merkel cells under confocal microscope.

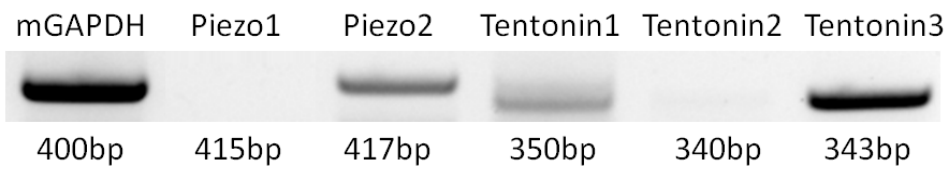



**Figure 2. Expression of Piezo2 and Tentonin3 mRNA in mouse Merkel cells**

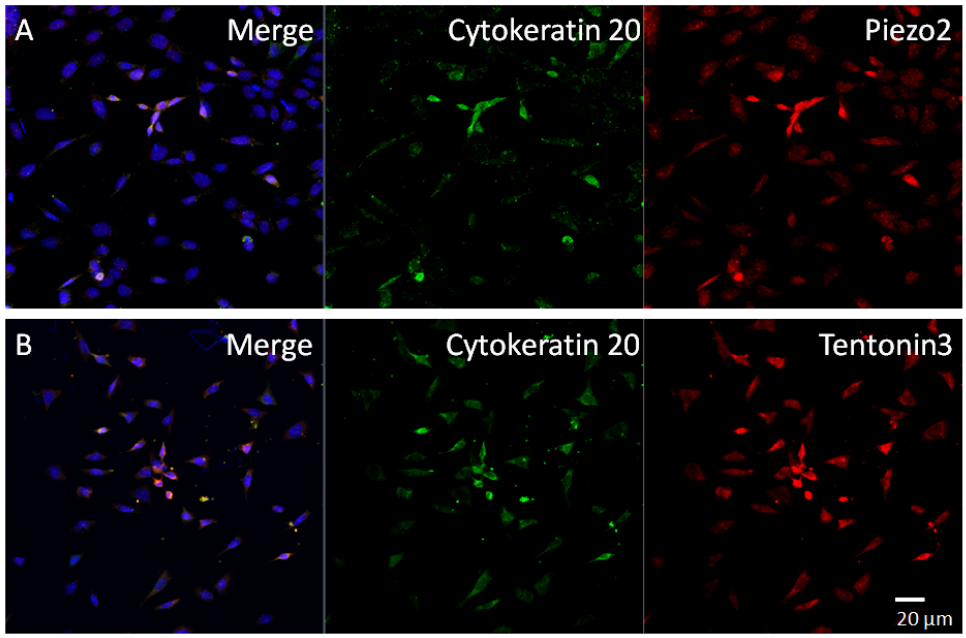



**Figure 3. Immunocytochemistry of cultured mouse Merkel cells**

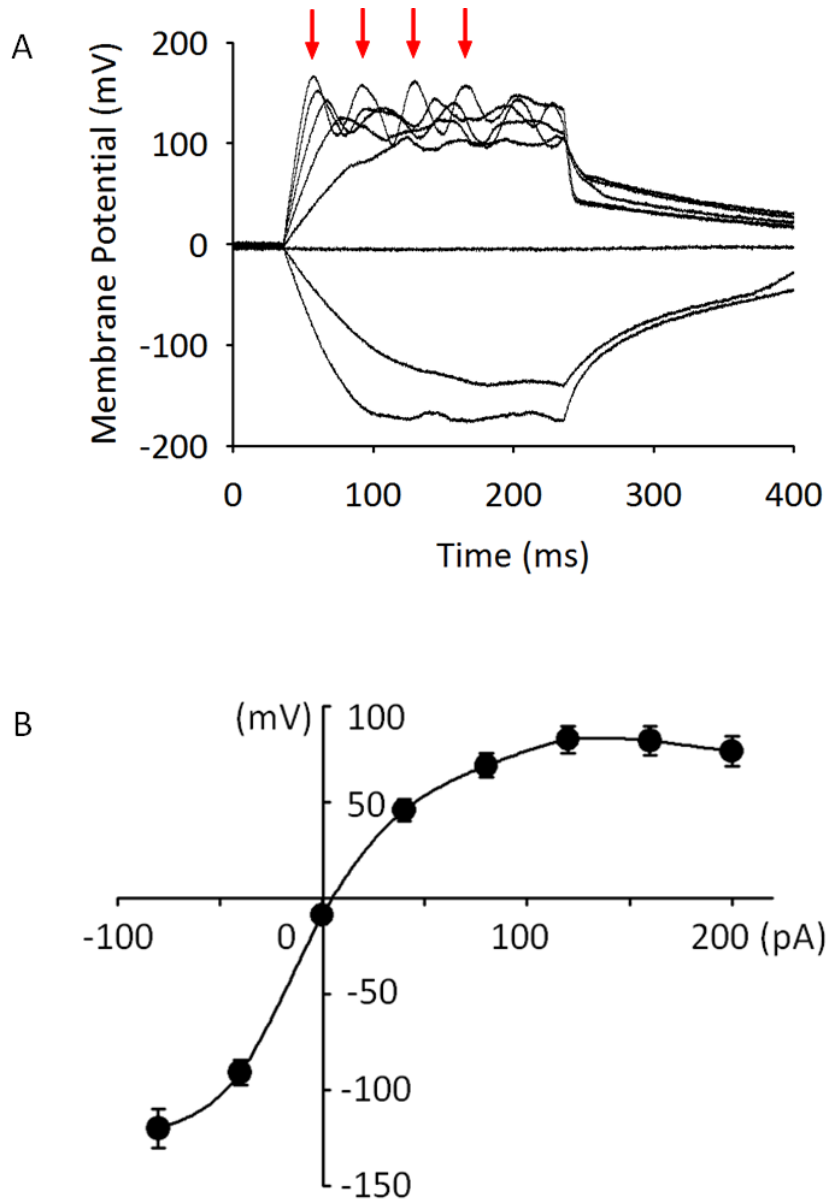



**Figure 4. Action potential firing in response to step current pulses in Merkel cells**

A. Representative traces of action potential responses (arrows) in Merkel cell.

B. Summary data of voltage-current relationships of Merkel cells (n = 39).

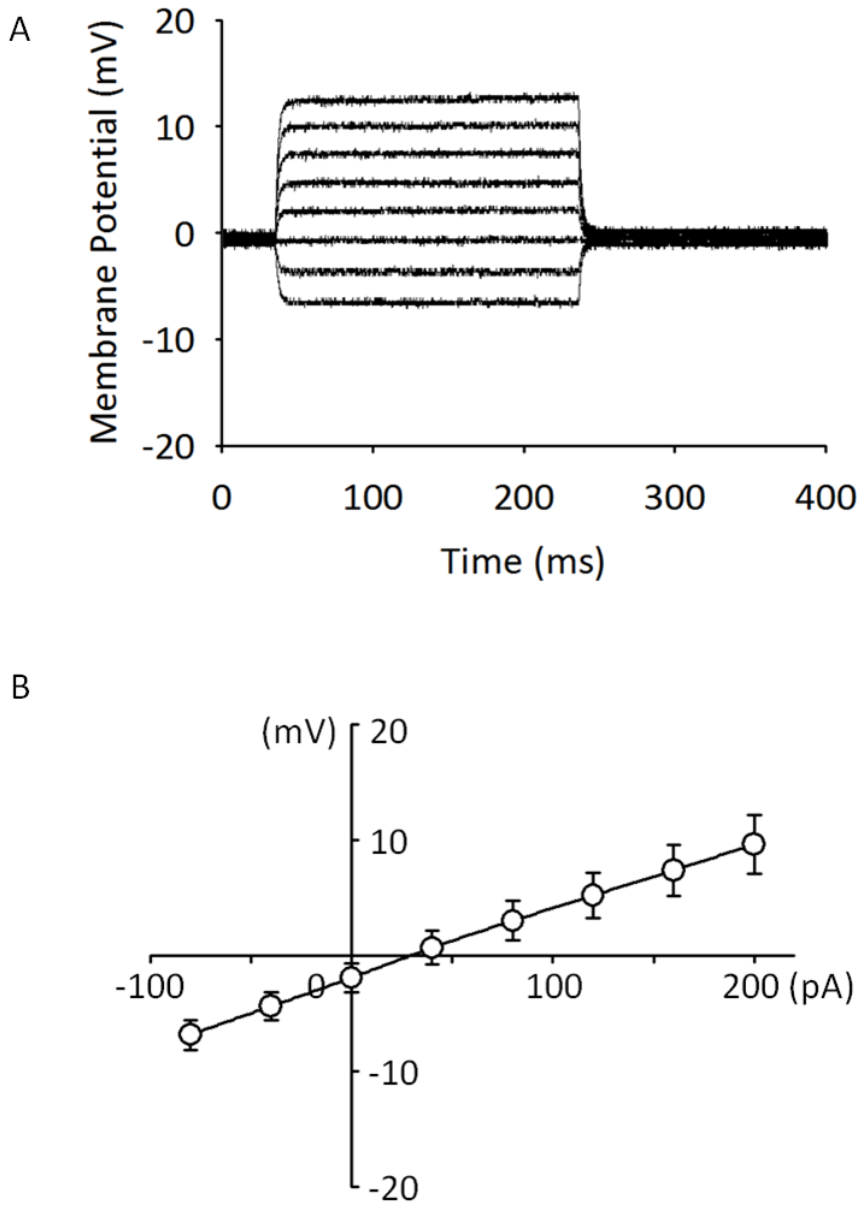
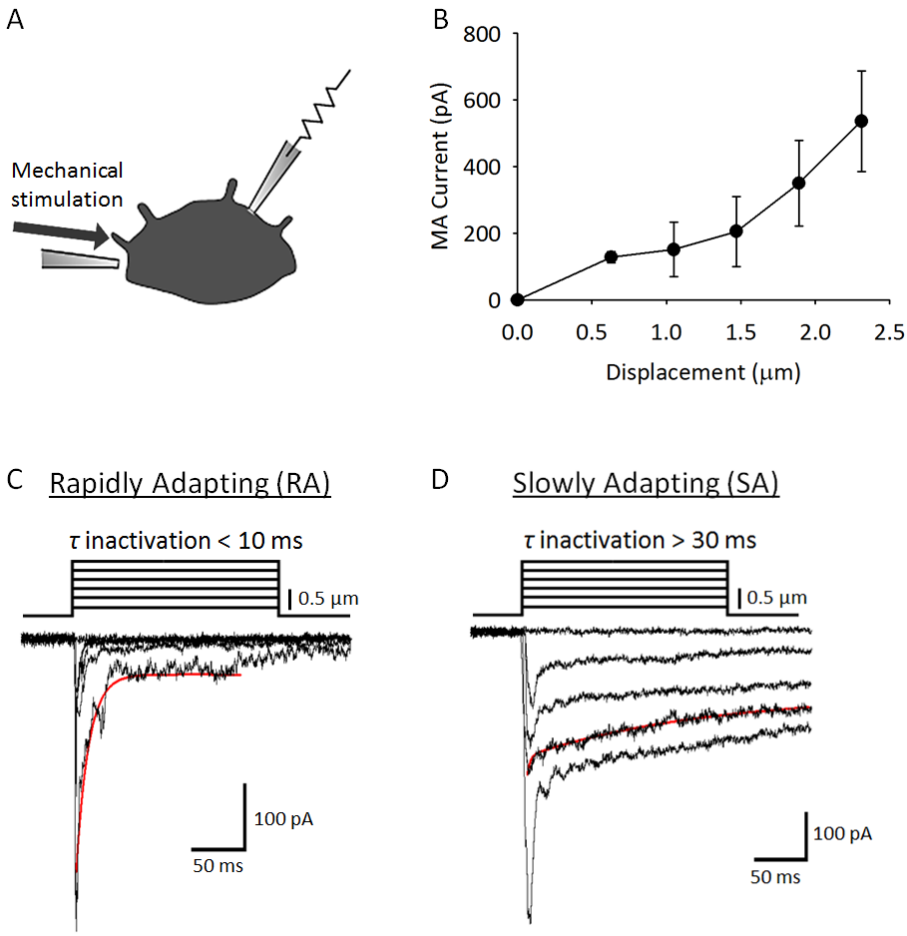



**Figure 5. No action potential firing in response to step current pulses in non-Merkel cells**

A. Representative traces of membrane responses to depolarizing current steps in a non-Merkel cell.

B. Summary data of voltage-current relationships of non-Merkel cells (n = 23).



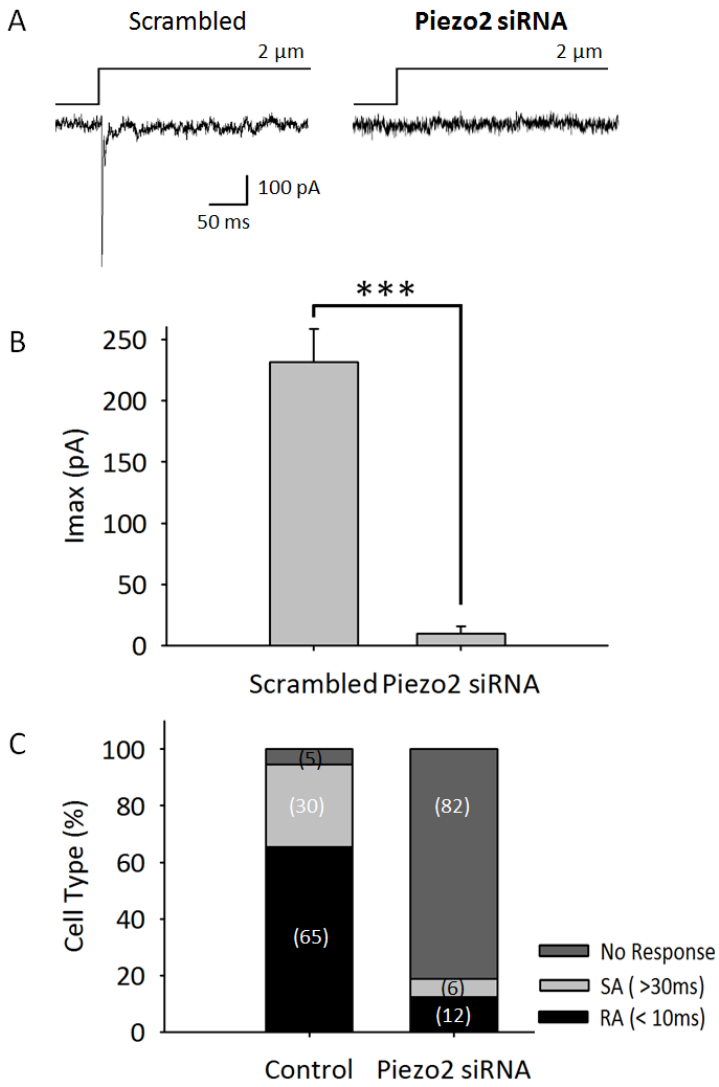

## Figure 6. Mechanically activated currents in Merkel cells

A. Schematic illustration of whole-cell patch configuration with mechanical stimulation through piezo-driven glass probe.

B. Summary data of average MA currents amplitude at different displacement (n = 31).

C. Representative traces of rapidly adapting MA currents from Merkel cells ($V_H$ = - 60 mV). Inactivation time were estimated from single-exponential fits.

D. Representative traces of slowly adapting MA currents from Merkel cells ($V_H$ = - 60 mV). Inactivation time were estimated from bi-exponential fits.




## Figure 7. Suppression of MA currents in Merkel cells by Piezo2 siRNA

A. Representative MA currents in Piezo2 siRNA transfected Merkel cells.

B. Average maximal amplitude of MA currents in Merkel cells transfected with scrambled siRNA (left, n = 38) or Piezo2 siRNA (right, n = 17). Bar represent the mean ± SE. Asterisks indicate statistically significant differences, *** $p < 0.001$, unpaired t test.

C. Proportion of Merkel cells transfected with scrambled siRNA (Control) or Piezo2 siRNA that respond to mechanical stimulation, with MA currents characterized by their inactivation time.

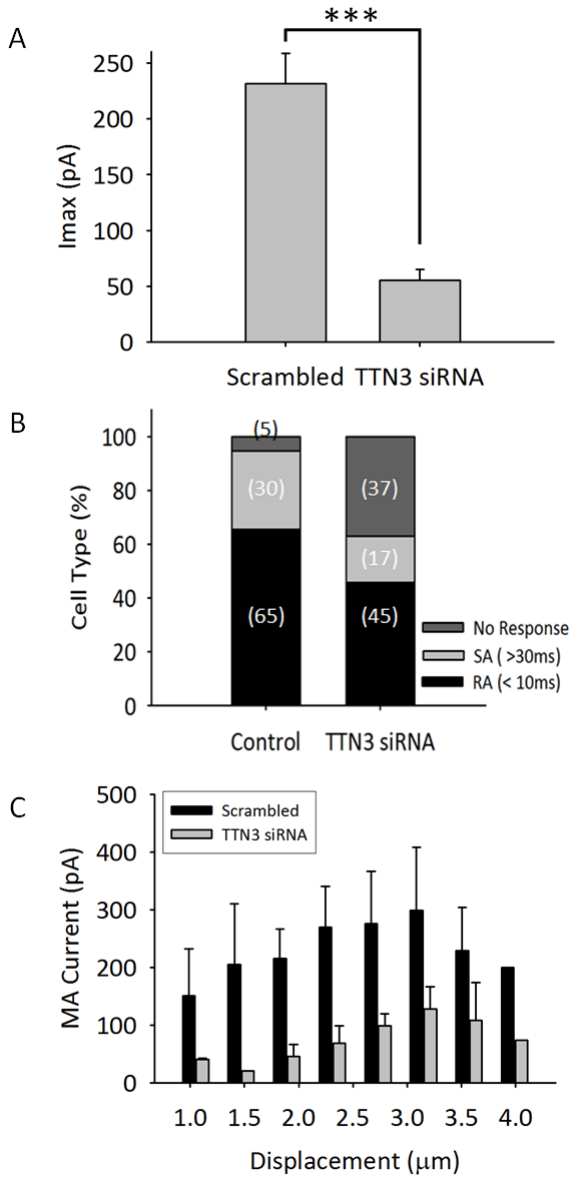
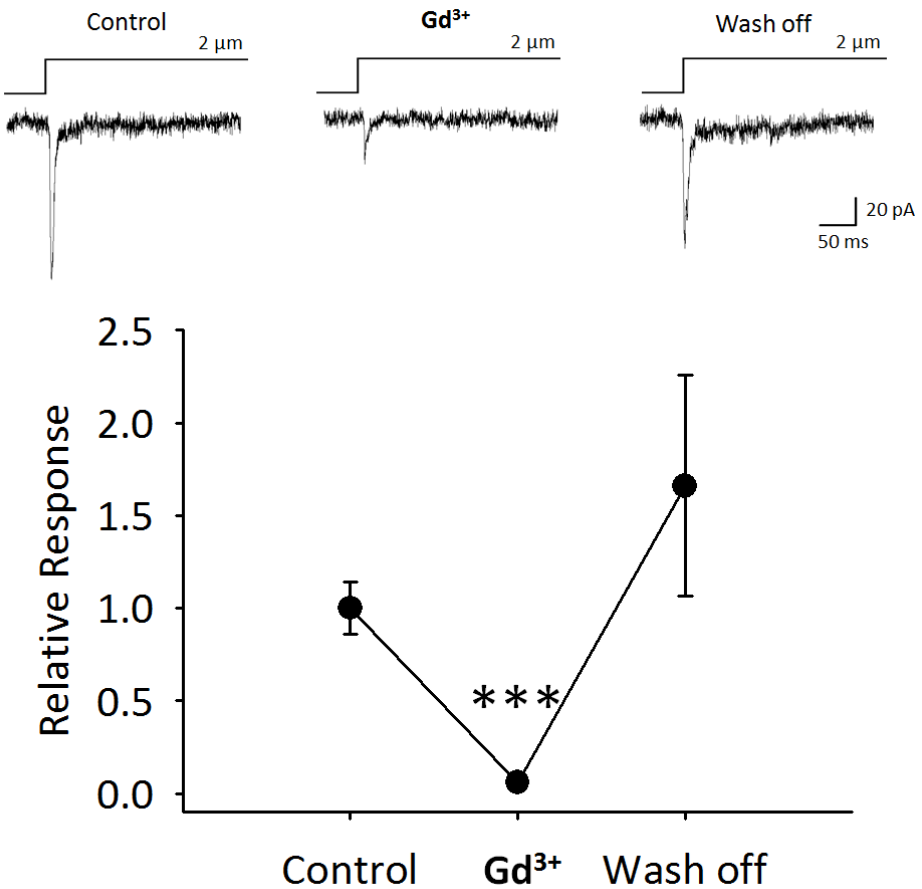



**Figure 8. Suppression of MA currents in Merkel cells by Tentonin3 siRNA**

A. Average maximal amplitude of MA currents in Merkel cells transfected with scrambled siRNA (left, n = 38) or Tentonin3 siRNA (right, n = 48). Bar represent the mean ± SE. Asterisks indicate statistically significant differences, *** $p < 0.001$, unpaired t test.

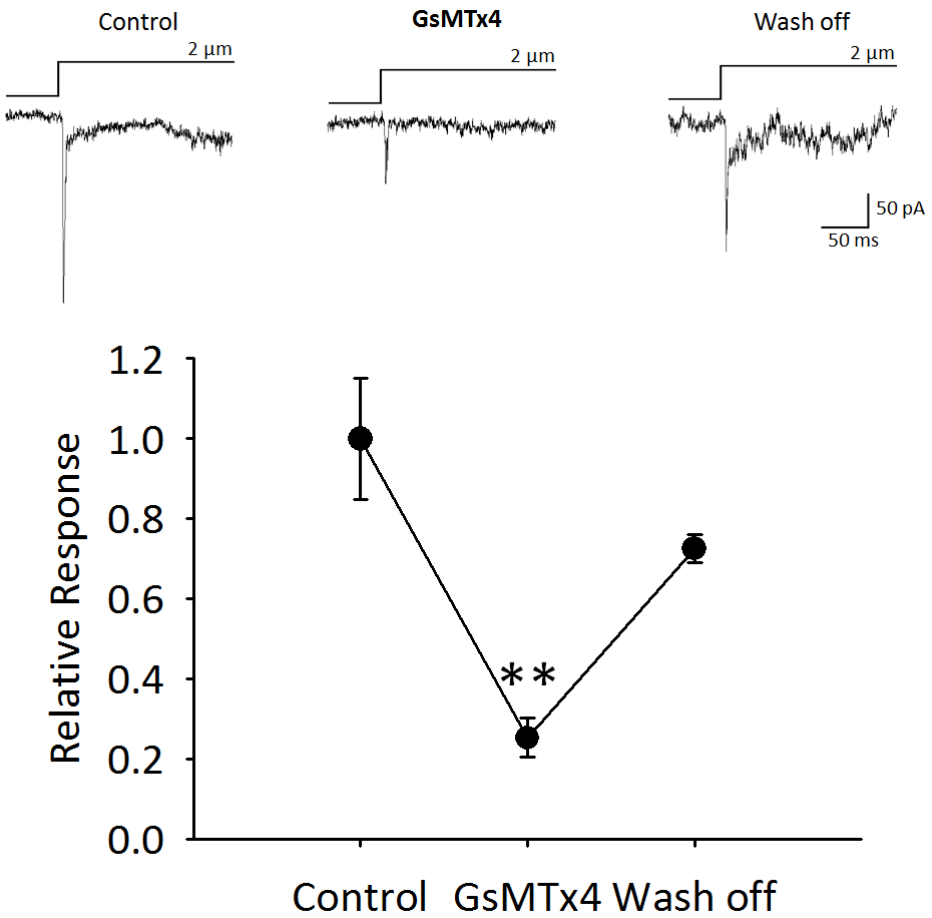
B. Proportion of Merkel cells transfected with scrambled siRNA (Control) or Tentonin3 siRNA that respond to mechanical stimulation, with MA currents characterized by their inactivation time.

C. Average maximal amplitude of MA currents in Merkel cells transfected with scrambled siRNA (black) or Tentonin3 siRNA (grey) at different displacement.




## Figure 9. Gadolinium inhibits MA currents in Merkel cells

MA currents in Merkel cells were inhibited by 100 µM Gadolinium ($Gd^{3+}$). After wash off, MA currents were recovered. Asterisks indicate statistically significant differences, *** $p < 0.001$, unpaired t test.




## Figure 10. GsMTx4 inhibits MA currents in Merkel cells

MA currents in Merkel cells were inhibited by 2.5 µM GsMTx4. After wash off, MA currents were recovered. Asterisks indicate statistically significant differences, ** $p < 0.01$, unpaired t test.

# DISCUSSION

The present study was designed to establish culture condition of Merkel cells and to characterize mechanosensitive currents in Merkel cells and finally to investigate which ion channels are related to mechanotransduction in Merkel cells. With our continuing efforts on researching for new ion channels, our research group found possible new ion channel that might involve in mechanotransduction and named it Tentonin3. In this study, we identified function of Tentonin3 in mechanotransduction in Merkel cells.

We modified culture condition of Merkel cells from mouse whisker hair follicles from previous studies (Fukuda, 1996; Cha et al., 2011). The critical step in Merkel cell culture was changing culture media after 30 minutes of pre-incubation. Without this process, we observed too much non-Merkel cells grew simultaneously in the dish.

We found that Merkel cells are excitable cells by depolarizing current steps patch clamp technique. However, non-Mekel cells never fired membrane action potentials by depolarizing current steps. This result is consistent with previous experimental studies in rats (Ikeda et al., 2014).

Moreover, mechanical stimulation induced mechanically activated (MA) currents in Merkel cells. There were two types of MA currents evoked in Merkel cells by mechanical stimulation. We characterized MA currents in Merkel cells by their tau inactivation time value. If MA currents have $\tau$-inactivation time less than 10 ms we called it rapidly adapting (RA) currents and slowly adapting (SA)

currents for τ-inactivation time more than 30 ms. Rapidly adapting currents in Merkel cells are consist with previous studies (Ikeda et al., 2014; Woo et al., 2014). However, slowly adapting currents in Merkel cells have not been reported and need more experiments and research to identify. In this present study we focused on Tentonin3, which might have important role in slowly adapting MA currents in Merkel cells.

It is known that knockdown of Piezo2 reduces MA currents in DRG sensory neurons (Coste et al., 2010). Piezo2 is strongly expressed in DRG sensory neurons. Also, it have been reported that Piezo2 is require for MA currents in Merkel cells (Woo et al., 2014). Through RT-PCR analysis and immunostaining method, we identified presence of Piezo2 in cultured Merkel cells. By Piezo2 siRNA transfection, we found significant reduction of MA currents in Merkel cells, which is consistent with previous study.

MA currents in Merkel cells are also reduced by Tentonin3 siRNA transfection. Interestingly, reduction of slowly adapting currents were much more than rapidly adapting currents compare to Piezo2 or scrambled siRNA transfected Merkel cells. These findings indicate that Tentonin3 ion channels plays role as the slowly adapting mechanotransducer in Merkel cells.

In conclusion, Merkel cells isolated from mice whisker hair follicles showed mechanically activated currents. Merkel cells display mixed MA currents showing rapidly adapting and slowly adapting currents. We confirmed that those currents were MA currents since they were inhibited by Gadolinium ($Gd^{3+}$) and GsMTx4. Piezo2 ion channels are required for MA currents in Merkel cells. Finally, Tentonin3 has potential roles in MA currents in Merkel cells, especially in slowly adapting

currents. This study on mechanosensitive currents in Merkel cells will provide basic information of Tentonin3 and more research remains to be done to understand function of Tentonin3.

# REFERENCES


1. Woo, S.H., et al., *Piezo2 is required for Merkel-cell mechanotransduction.* Nature, 2014. 509(7502): p. 622-6.
2. Woo, S.H., E.A. Lumpkin, and A. Patapoutian, *Merkel cells and neurons keep in touch.* Trends Cell Biol, 2015. 25(2): p. 74-81.
3. Ranade, S.S., et al., *Piezo2 is the major transducer of mechanical forces for touch sensation in mice.* Nature, 2014. 516(7529): p. 121-5.
4. Maksimovic, S., et al., *Epidermal Merkel cells are mechanosensory cells that tune mammalian touch receptors.* Nature, 2014. 509(7502): p. 617-21.
5. Coste, B., et al., *Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels.* Science, 2010. 330(6000): p. 55-60.
6. Lumpkin, E.A. and M.J. Caterina, *Mechanisms of sensory transduction in the skin.* Nature, 2007. 445(7130): p. 858-65.
7. Haeberle, H., et al., *Molecular profiling reveals synaptic release machinery in Merkel cells.* Proc Natl Acad Sci U S A, 2004. 101(40): p. 14503-8.
8. Piskorowski, R., et al., *Voltage-activated ion channels and Ca(2+)-induced Ca (2+) release shape Ca (2+) signaling in Merkel cells.* Pflugers Arch, 2008. 457(1): p. 197-209.
9. Maksimovic, S., Y. Baba, and E.A. Lumpkin, *Neurotransmitters and synaptic components in the Merkel cell-neurite complex, a gentle-touch receptor.* Ann N Y Acad Sci, 2013. 1279: p. 13-21.

10. Fukuda, J., *A pure, monolayer culture of Merkel cells from sinus hair follicles of the rat.* Neurosci Lett, 1996. 216(2): p. 73-6.

11. Ma, Q., *Merkel cells are a touchy subject.* Cell, 2014. 157(3): p. 531-3.

12. Zimmerman, A., L. Bai, and D.D. Ginty, *The gentle touch receptors of mammalian skin.* Science, 2014. 346(6212): p. 950-4.

13. Schrenk-Siemens, K., et al., *PIEZO2 is required for mechanotransduction in human stem cell-derived touch receptors.* Nat Neurosci, 2015. 18(1): p. 10-6.

14. Ikeda, R., et al., *Merkel cells transduce and encode tactile stimuli to drive Abeta-afferent impulses.* Cell, 2014. 157(3): p. 664-75.

15. Ikeda, R. and J.G. Gu, *Piezo2 channel conductance and localization domains in Merkel cells of rat whisker hair follicles.* Neurosci Lett, 2014. 583: p. 210-5.

16. Cha, M., et al., *Shear mechanical force induces an increase of intracellular Ca2+ in cultured Merkel cells prepared from rat vibrissal hair follicles.* J Neurophysiol, 2011. 106(1): p. 460-9.

# 국 문 초 록

Merkel 세포는 신경 섬유가 밀집해 있으며, 피부 신경 내분비를 담당하는 상피성 촉각 세포이다. 역학적 신호의 인지 과정에 관여하고 촉각을 수용한다. 그 이름은 1875년 표피 아래쪽에서 새로운 유형의 세포를 발견하였던 프리드리히 지크문트 메르켈의 이름에서 유래되었다. 이 세포는 촉각이 예민한 부위에 존재하는 표피의 기저층에 위치해 있고 몸의 특정 부위에만 존재한다. 신경말단과 시냅스로 접촉하여 Merkel cell-neurite 복합체를 형성한다. Merkel cell-neurite 복합체는 light touch 나 hair movement를 기계적 자극으로 변환한다고 알려져 있다. 하지만 Merkel 세포가 어떻게 촉각을 전달하는지 그 분자 역학적 기전은 아직 명확하게 밝혀져있지 않다. 최근 연구들에 의하면 Piezo 이온 채널들인 Piezo1과 Piezo2 이온 채널들이 mechanically activated ion channel (MA) 로 밝혀졌다. 특히 Dorsal root ganglion (DRG)에 분포하고 있는 Piezo2 이온 채널들은 mechanotransduction에 관여하고 있다는 것이 연구를 통해 밝혀졌다. 하지만 촉각을 감지하는 데 있어서 Piezo2가 유일한 이온 채널인 것인지, 다른 분자적 물질들이 관여하고 있는지는 아직 확인되어지지 않은 상황이다. 따라서 본 연구에서는 어떻게 Merkel 세포가 촉각을 전달하는지, 어떠한 분자들이 전달에 관여하는지 알아보고자 하였다.

마우스 수염 모낭에 붙은 Merkel 세포를 배양한 후, 패치 클램프를 사용하여 Merkel 세포에 기계적 자극을 주었을 때 나타는 전류를 측정하

였다. 이 전류는 곧 기계 채널에 의한 전류로서 Merkel 세포가 촉각을 전달하는데 중요한 역할을 한다는 것을 알 수 있었다. 이 전류가 기계적 자극에 의한 전류임은 Gadolinium과 GsMTx4 화학물질의 처리에 의해 전류가 사라지는 것으로 보아 확인 할 수 있었다.

본 연구단에서는 새로운 기계 채널을 찾기 위한 스크리닝을 통하여 찾은 새로운 채널을 'Tentonin3' 라고 칭하였다. Merkel 세포에서 Tentonin3의 발현을 저해시킨 후 기계적 자극에 의한 전류를 측정해보았을 때, slowly adapting 전류의 크기가 크게 줄어드는 것을 확인할 수 있었다. 따라서 이 결과는 Tentonin3가 기계적 자극의 의한 전류 발생에 중요한 역할을 할 것이라는 가능성을 시사한다.

주요어: 메르켈 세포, Piezo2, Tentonin3, 촉각, 기계채널

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