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学 位 論 文 題 目　Interaction between TRPV3 and TMEM79 in mouse keratinocytes and its physiological significance

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Summary of Doctoral Thesis

Lei, Jing

Interaction between TRPV3 and TMEM79 in mouse keratinocytes and its physiological significance

TRPV3, a warmth-activated (> 33℃) cation-permeable transient receptor potential (TRP) ion channel, is predominantly expressed in skin keratinocytes and participates in various physiological processes ranging from somatosensation to inflammation. Although some progress has been made in understanding TRPV3, numerous issues remain to be addressed. First, modulation of ion channels could affect their subcellular localization, channel properties, or gating, which is considered important for physiological changes in organisms. Despite TRPV3 being activated by a wide range of external stimuli and its channel activities being regulated by several intracellular factors, little is known about TRPV3-associated proteins. Furthermore, although TRPV3 knockout mice exhibit a defective preference to warm temperature, it remains controversial whether TRPV3 directly contributes to thermosensation. Moreover, expression levels of TRPV3 are increased in isolated keratinocytes from patients with atopic dermatitis (AD), a common chronic skin disease associated with inflammation, itch, and skin barrier dysfunction. Multiple TRPV3 gain-of-function mutations are also associated with a hereditary skin disease, olmsted syndrome (OS), characterized by abnormal keratinization and severe itch. However, the underlying mechanism by which TRPV3 is involved in such cutaneous diseases is still poorly understood. Interestingly, transmembrane protein 79 (TMEM79), a putative five-pass transmembrane protein, is similarly highly expressed in skin keratinocytes. TMEM79 is associated with skin barrier formation and pathogenesis in AD-like mice. Therefore, I proposed the

straightforward hypothesis that both TRPV3 and TMEM79 are likely to function together in the skin.

In this study, I first evaluated whether TMEM79 could modulate TRPV3-mediated currents by performing whole-cell patch-clamp experiments and found that mouse TMEM79 was capable of suppressing 2-aminoethoxy diphenyl borate (2-APB)-induced current amplitudes in HEK293 cells transiently expressing mouse TRPV3. In line with this, further biotinylation assays and co-immunoprecipitation revealed that TMEM79 could decrease the expression levels of plasma membrane TRPV3 through a physical interaction. In addition, with co-expression of TMEM79, immunofluorescence staining with different organelle markers showed that TRPV3 was largely retained in the endoplasmic reticulum (ER). Meanwhile, some TRPV3 was localized within lysosomes, suggesting that TMEM79 promoted TRPV3 degradation through the lysosomal pathway, which is consistent with a reduction in TRPV3 total protein levels as determined by Western blot. Taken together, these findings indicate that TMEM79 inhibits the membrane trafficking of TRPV3 while simultaneously facilitating the degradation of TRPV3, thereby affecting TRPV3-related activities.

Consistent with the results in HEK293 cells, 2-APB-induced TRPV3 currents were larger in primary mouse keratinocytes lacking TMEM79. This was supported by subsequent Ca^{2+} imaging in which the application of either a cocktail 2-APB/carvacrol or heat resulted in larger Ca^{2+} influxes in keratinocytes from TMEM79$^{-/-}$ mice than from wild-type mice. Taken together, these findings demonstrate that TMEM79 is required for the regulation of TRPV3 channels in mouse primary keratinocytes, implying a potential physiological interaction *in vivo*.

To understand the significance of the interaction between TRPV3 and TMEM79 *in vivo*,

I first sought to confirm that TRPV3 played a role in warmth sensing by conducting a behavioral assay using a thermal gradient ring. Surprisingly, I found that TMEM79$^{-/-}$ mice displayed a strong preference for warmer temperatures compared to wild-type and TRPV3$^{-/-}$ mice. This is likely caused by higher endogenous TRPV3 protein levels in keratinocytes when TMEM79 is deleted. These findings suggest that TMEM79 may be involved in temperature sensation through the modulation of TRPV3 channel density in the plasma membrane of skin keratinocytes.

Together, this project reveals a novel interaction between TRPV3 and TMEM79, in which TMEM79 functions as a modulator of TRPV3 by affecting TRPV3 trafficking. Dysfunction of TRPV3 primarily contributes to skin physiology and pathophysiology. Therefore, this interaction provides additional insight into our overall understanding of skin pathogenesis. As a future vision, the modulation of cell surface TRPV3 may represent a valuable therapeutic approach for the treatment of skin diseases. Of particular therapeutic importance, channel activity can be modulated by the number of ion channels present. Based on studies by myself and others, TMEM79 is likely to function as an enzyme, and it is possible that we can indirectly manage TRPV3 activity for the treatment of skin conditions by regulating TMEM79.

Given that TRPV3 is activated by human body temperature, it is proposed to be a thermosensor in our skin by conveying temperature information from keratinocytes to the brain. However, the mechanism by which an organism orchestrates thermotransduction in the skin needs further investigation. Modulation of TRPV3 by its associated proteins such as TMEM79 could be one intriguing approach for further elucidating these mechanisms.

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Results of the doctoral thesis screening

博士論文審査結果

Name in Full
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Title
論文題目　Interaction between TRPV3 and TMEM79 in mouse keratinocytes and its physiological significance

TRPV3 は温かいと感じられる温度域で活性化されるカチオンチャネルである。TRPV3 は主として表皮細胞に発現しており、皮膚の恒常性に重要な役割を果たすと考えられているが、TRPV3 の機能や活性の制御機構にはいまだ不明な点が多く、TRPV3 に相互作用する分子もほとんど知られていない。出願者は、表皮細胞に多く発現する膜タンパク質 TMEM79 の遺伝子欠失マウスが TRPV３変異マウスとよく似た皮膚炎を起こすとの報告に着目し、TRPV3 と TMEM79 が機能的に連関するとの仮説をたててその検証を行った。まず、HEK293 細胞に TRPV3 を単独で発現、あるいは TMEM79 と共発現させて全細胞パッチクランプ解析を行うことにより、TRPV3/TMEM79 共発現細胞では TRPV3 単独発現細胞と比較して TRPV3 アゴニストである 2-aminoethoxy diphenyl borate (2-APB)により惹起される電流が小さいことを見出した。さらにこれらの細胞を生化学的および形態学的に解析した結果、TRPV3/TMEM79 共発現細胞では、TRPV3 単独発現細胞と比較して、細胞膜上の TRPV3 タンパク質量が著しく減少すること、TRPV3 と TMEM79 が共沈することを明らかにした。これらの結果は、TMEM79 が TRPV3 との相互作用を介して細胞膜上の TRPV3 の発現量を下げることにより TRPV3 の活性を負に制御することを示唆する。次に出願者は、マウスの尾から単離した表皮ケラチノサイトの全細胞パッチクランプ解析において、皮膚に発現する TRP チャネルである TRPV3 および TRPV4 それぞれのアゴニストである 2-APB および GSK101 を加えて発生する電流を測定することにより、84%の細胞が 2-APB と GSK101 の両方に応答すること、すなわち TRPV3 よ TRPV4 を共発現することを確認した。そして、この実験系を適用することにより TMEM79 遺伝子欠失マウスから単離したケラチノサイトでは、2-APB により惹起される電流だけがコントロールより大きいことを明らかにした。さらに、Thermal Gradient Ring を用いた成体マウスの行動から、野生型マウスに比べて TRPV3 遺伝子欠失マウスでは温度選択性が低い温度にシフトするのに対し、TMEM79 遺伝子欠失マウスでは逆に高い温度シフトすることを見出した。この結果は、TMEM79 が TRPV3 の活性を負に制御することを示した培養細胞における観察とも整合する。

以上のように、出願者は、表皮ケラチノサイトにおいて TRPV3 の相互作用分子として TMEM79 を見出し、TMEM79 が TRPV3 の動態に影響を与えることによりその活性を制御することを明らかにした。本論文は、皮膚において TRPV3 が関与すると考えられる温度や痒みの受容のメカニズムを解明するうえで重要な知見を与えるものであり、今後の当該分野の発展に資すると考えられる。したがって、審査委員全員が本論文は学位論文として相応しいものであると判断した。