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学位(専攻分野)	博士(理学)
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学 位 論 文 題 目	Physiological significance of TRPV4 channels in mouse Schwann cells
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(Form 3)

Summary of Doctoral Thesis

Name in full: Feng, Xiaona

Title: Physiological significance of TRPV4 channels in mouse Schwann cells

Schwann cells (SCs) are the primary glial cells of the peripheral nervous system. Myelinating SCs wrap around large-diameter axons and form the myelin sheath. While many transcription factors and signaling molecules are involved in SC myelination, calcium signaling has been found to be an important mediator of this process. Transient receptor potential vanilloid 4 (TRPV4), a member of the TRP channel family, is a non-selective calcium-permeable cation channel. TRPV4 is expressed and activated throughout the body by various stimuli including mechanical stimulation, moderate heat, osmolarity and some endogenous or exogenous chemicals. According to recent reports, this channel was found to be widely expressed and functional in various glial cells, including astrocytes, microglia, oligodendrocytes and satellite glial cells. However, whether TRPV4 is expressed and functional in SCs or not remains unclear.

To clarify the expression and function of TRPV4 in SCs, I isolated and purified SCs from the sciatic nerves of postnatal and adult mice. Both TRPV4 mRNA and protein were detected in the purified SCs by RT-PCR and western blot analyses, which indicates that TRPV4 is expressed in cultured SCs. In addition, TRPV4-mediated responses to 1.0 μM GSK1016790A, a TRPV4 selective agonist, were observed using both calcium-imaging and whole-cell patch-clamp methods. These demonstrate the functional expression of TRPV4 in cultured SCs.

Furthermore, TRPV4 was found to be expressed in sciatic nerves *in vivo* by western blot. However, I did not observe any differences in the expression levels of the key myelin structural proteins such as myelin-associated glycoprotein (MAG),

myelin protein zero (P0) or myelin basic protein (MBP) between WT and TRPV4KO mice by immunostaining and western blot analysis, suggesting that TRPV4 is not involved in normal myelin development in mice. However, after sciatic nerve cut injury, TRPV4 expression gradually increased with sciatic nerve demyelination, even under conditions without macrophages which are known to express TRPV4. This suggests that the increase in TRPV4 after sciatic nerve injury is mainly derived from SCs. Furthermore, I confirmed that TRPV4 is expressed in unmyelinating SCs, but not in myelinating SCs by double immunostaining of TRPV4 with glial fibrillary acidic protein (GFAP) or MBP in teased sciatic nerves, and that unmyelinating SCs were increased after nerve injury. These results suggest why TRPV4 was increased after injury. I next examined whether the increased TRPV4 is active under physiological conditions. I measured the temperature-evoked increase in intracellular calcium concentrations using a calcium-imaging method in cultured SCs. TRPV4-dependent intracellular calcium increases were clearly observed in the unmyelinating SCs under body temperature, which suggests that TRPV4 is constitutively active in SCs at normal body temperature.

To determine whether the recovery process from sciatic nerve cut-induced Wallerian degeneration is affected by the increase in TRPV4 expression, I created a sciatic nerve cut injury model in both WT and TRPV4KO mice. Interestingly, western blot analysis showed significantly higher levels of P0, MAG and MBP proteins in TRPV4KO mice compared with WT mice 7 days after injury. In addition, I examined the function of sciatic nerves of these mice by walking track analysis 2 months after injury. Sciatic functional index (SFI) values were significantly smaller in TRPV4KO mice than in WT mice, indicating that the absence of TRPV4 impairs the functional recovery of sciatic nerves after injury. To further evaluate the regeneration of sciatic nerves, the structure of the distal stumps from these mice was analyzed using an electron microscope (EM). I found the reformed myelin was significantly thinner in

TRPV4KO mice than in WT mice at 2 months after sciatic nerve injury. However, Walking track analysis and EM analysis revealed that the sciatic nerve function and the remyelination were similarly recovered by 6 months both in between WT and TRPV4KO mice after injury. these results demonstrate that the lack of TRPV4 delayed sciatic nerve remyelination and functional recovery following sciatic nerve cut-induced Wallerian degeneration.

In conclusion, TRPV4 is functionally expressed in mouse SCs and is involved in remyelination after sciatic nerve cut injury.

Form8 : Separate Sheet
(様式8・別紙)

Results of the doctoral thesis screening

博士論文審査結果

Name in Full
氏　　名　　Feng, Xiaona

Title
論文題目　　Physiological significance of TRPV4 channels in mouse Schwann cells

シュワン細胞は末梢神経系で神経軸索を包み、髄鞘形成による跳躍伝導に関与している。中枢神経系で同様の役割を担うオリゴデンドロサイトには温度感受性 TRPV4 チャネルが発現してその機能に関わることが知られているが、シュワン細胞における TRPV4 の発現・機能は報告がない。本論文は、野生型マウスと TRPV4 欠損マウスの坐骨神経傷害モデルを比較して坐骨神経傷害による脱髄からの回復過程への TRPV4 の関与を明らかにした論文である。

出願者は、野生型マウス坐骨神経からシュワン細胞を単離培養して種々の TRP チャネルの遺伝子発現および機能を解析して TRPV4 の遺伝子およびタンパク質の発現と Ca イメージング法およびパッチクランプ法による TRPV4 機能を観察した。シュワン細胞での 37 度への温度上昇による細胞内 Ca 濃度増加も確認した。シュワン細胞の 3 つの髄鞘構造タンパク質、P0 (protein zero), MBP (myelin basic protein), MAG (myelin-associated glycoprotein)の発現を野生型と TRPV4 欠損の 15 週齢マウスで比較したが、差が確認できなかったことから、シュワン細胞の正常発達過程に TRPV4 は関与しないと考えられた。そこで、坐骨神経傷害モデルで検討した。切断 5 日目に神経軸索マーカーの NF160 は消失して 14 日目から再発現が観察された。一方、MAG, P0 は遅れて発現が減少し、切断後 21 日から発現が増加した。興味深いことに、非傷害コントロールでは TRPV4 タンパク質発現は観察されず、切断後 5 日～14 日に発現が増加していた。この発現増加は MAG, P0 の発現増加に先んじて起こっており、単離培養シュワン細胞で TRPV4 発現が観察されたのは、細胞単離手技による一時的な傷害を反映したものだと理解される。傷害後の坐骨神経サンプルの解析でも切断 7 日後に TRPV4 の発現増加が観察され、髄鞘形成を抑制する c-jun が発現増加し、促進する Krox10 が発現減少していたことは、TRPV4 の髄鞘形成への寄与が示唆される。事実、TRPV4 は髄鞘形成していないシュワン細胞でより多く発現していることが、発現解析から明らかとなった。TRPV4 欠損マウスの坐骨神経傷害モデルで切断 7 日後に P0, MBP, MAG の発現がより大きいことは、TRPV4 欠損マウスで傷害後の髄鞘クリアランスがより遅れていることを意味する。坐骨神経機能の指標となる切断後のマウス後肢の拘縮が TRPV4 欠損マウスでより著しく、切断後の電子顕微鏡観察による髄鞘厚がより小さいことはそれを支持する。マウス坐骨神経切断後にシュワン細胞による TRPV4 依存的な髄鞘断片のクリアランスと髄鞘再形成が起こっているものと結論した。

以上の結果は、TRPV4 はシュワン細胞における神経損傷後の髄鞘再形成に深く関与することを示した画期的な知見であり、その生理学的意義は極めて大きいことから、全会一致で合格と判定した。