

# Activation of the Transient Receptor Potential Ion Channel TRMP8 Mediates Upregulation Of Profibrotic Genes, A New Pathway to Tissue Fibrosis

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**Introduction:** Enhanced cold sensitivity is an early and consistent phenomenon in scleroderma (SSc). TRPM8 protein is cold- and menthol-sensing calcium (Ca<sup>2+</sup>) ion channel. In this study, we evaluated TRPM8 expression, the effects of TRPM8 activation on fibroblast (FB) fibrotic gene expression, and intracellular signaling.

**Methods:** FBs were isolated from involved SSc skin and matched control subjects. TRPM8 activation in FBs was triggered by the TRPM8 agonist menthol (MT) or by exposure of cells to cold (18°C). Intracellular calcium concentration ([Ca<sup>2+</sup>]<sub>i</sub>) was determined using Fura-2 or Fura-4. The mRNA and protein expression levels were determined by qPCR and WB. The production of ROS was detected by dihydroethidium (DHE). SMAD3 binding to the CTGF promoter region was detected by chromatin immunoprecipitation assay (ChIP).

**Results:** TRPM8 is expressed in dermal FBs. The expression levels of TRPM8 were significantly higher in SSc-FBs and SSc-skin biopsies. MT or cold exposure increased [Ca<sup>2+</sup>]<sub>i</sub>, enhanced expression of COL1A1, αSMA, FN, and CTGF, and also evoked production of intracellular ROS. SSc-FBs were more sensitive to MT or cold than normal FBs. These effects were blocked by the addition of Capsazepine, or TRPM8 siRNA, or antioxidants. Moreover, MT induced SMAD3 phosphorylation and nuclear accumulation. Chip assay confirmed that SMAD3 is recruited to the CTGF promoter after MT stimulation in FBs.

**Conclusion:** Functional TRPM8 is expressed in human dermal FBs and enhanced expression was observed in SSc FBs and skin. The activation of TRPM8 mediated enhanced expression of the profibrotic genes in FBs via the calcium-ROS-SMAD3 signaling pathway.