研究例1

・表皮細胞の角化制御

The impact of extracellular syntaxin4 on HaCaT keratinocyte behavior

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1. Introduction

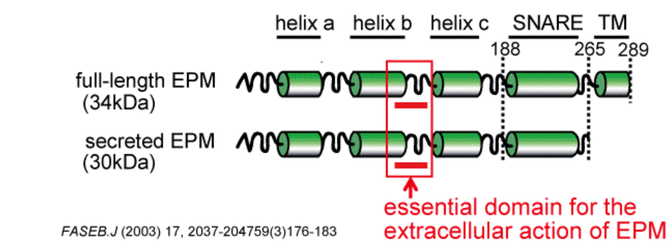
- Unsaturated fatty acid affects calcium influx in keratinocytes and triggers the epidermal hyperplasia with perturbation of the keratinization program.
- Epimorphin(EPM) is a stromal signaling factor that is temporally secreted via a non-classical route to regulate the morphogenesis of various epithelia, including skin epidermis.
- Calcium influx cues the extracellular secretion of EPM and perturbs its signaling gradient in the epidermis.
- An antagonistic circular peptide(EPn1) of EPM prevents function of EPM, so EPn1 prevents abnormal differentiation.
- EPM belongs to syntaxin family and syntaxinx4 (STX4) resembles to EPM.
- EPM expresses mainly in dermis, whereas STX4 strongly expresses in the epidermis.

Hypothesis

- STX4 participates in epidermal differentiation as EPM.
- The functional core of STX4 lies in the corresponding position to that of EPM.
- Antagonists of STX4 can be generated from the potent functional core of STX4, as for EPn1 from EPM.

2. EPM and EPn1 (back ground)

a) EPM is secreted after removal of C-terminal TM domein.



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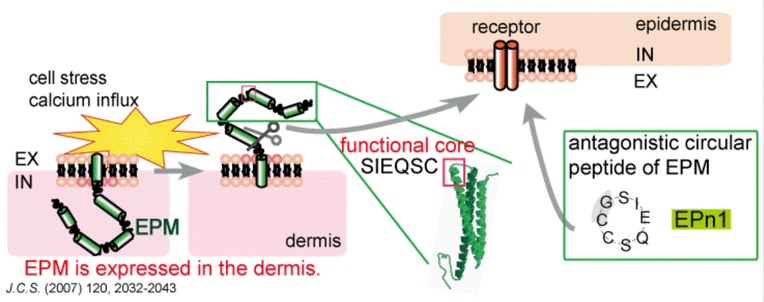
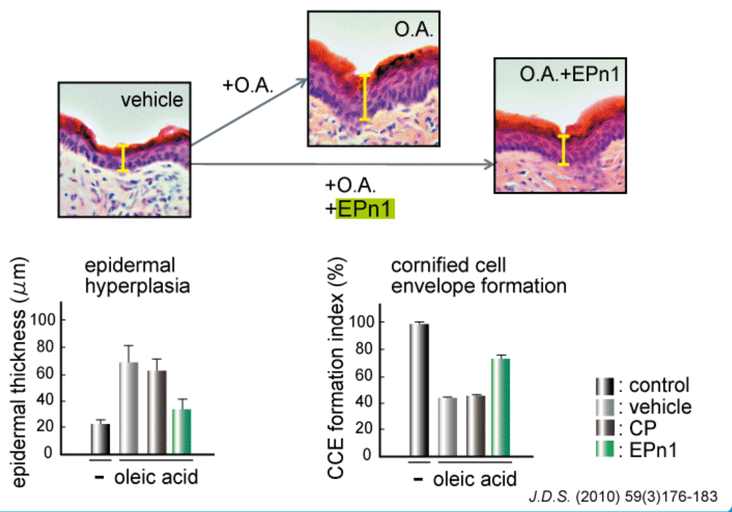
b) EPn1 prevents function of extracellularly secreted EPM.



J.C.S. (2007) 120, 2032-2043

c) Oleic acid (O.A.) leads to extracellular secretion of EPM, and the subsequent abnormal differentiation of epidermis, which was prevented by EPn1.



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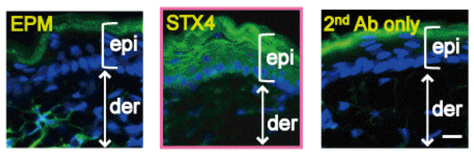
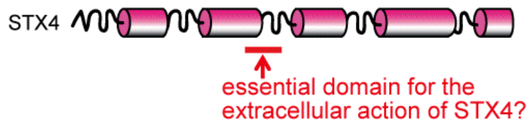
3. Expression of STX4 in the eidermis

a) STX4 was expressed both in the epidermis and dermis in skin.



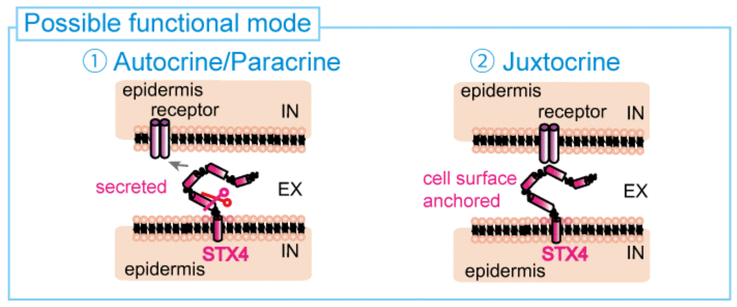
b) The predicted structure of STX4



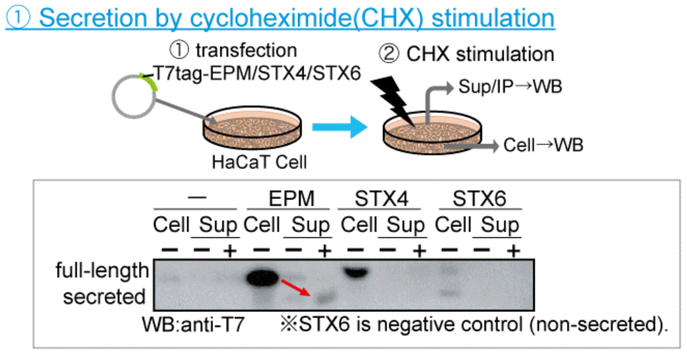
STX4 is resembles to EPM.
Homology; 40% (amino acid)

4. Extracellular localization of the subpopulation of STX4

Possible functional mode

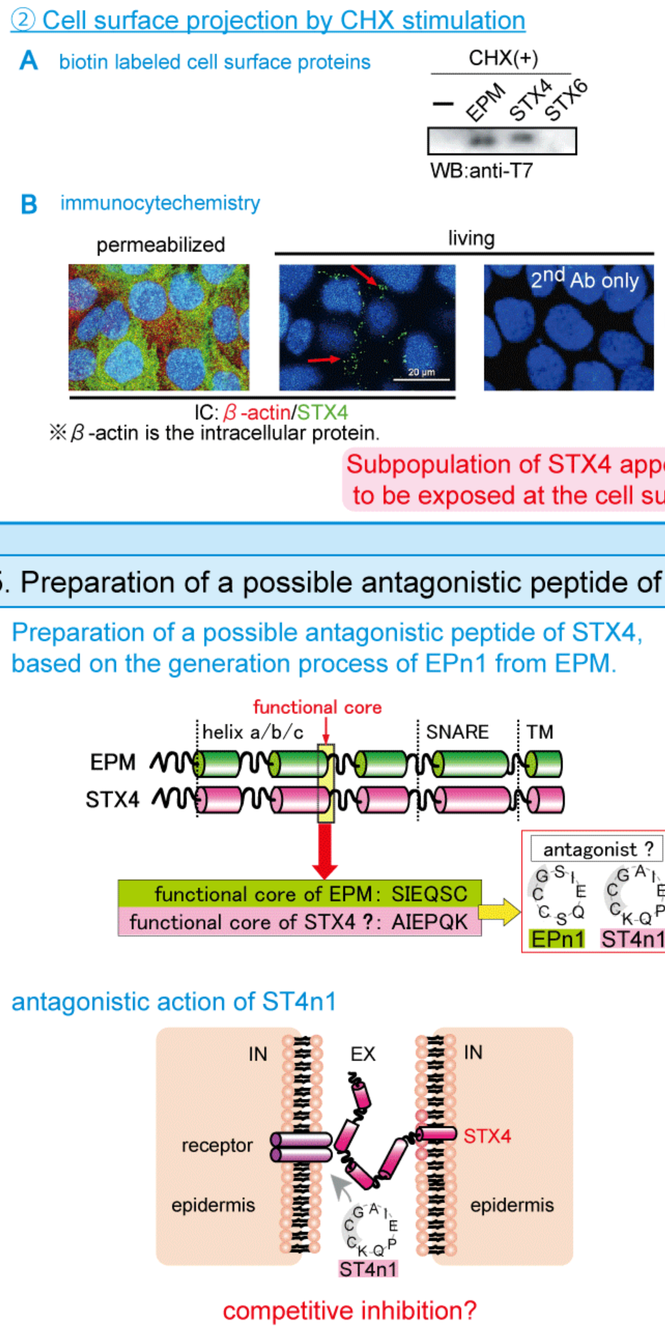


① Secretion by cycloheximide(CHX) stimulation



Secretion of STX4 was not detectable.

② Cell surface projection by CHX stimulation



Subpopulation of STX4 appeared to be exposed at the cell surface.

5. Preparation of a possible antagonistic peptide of STX4

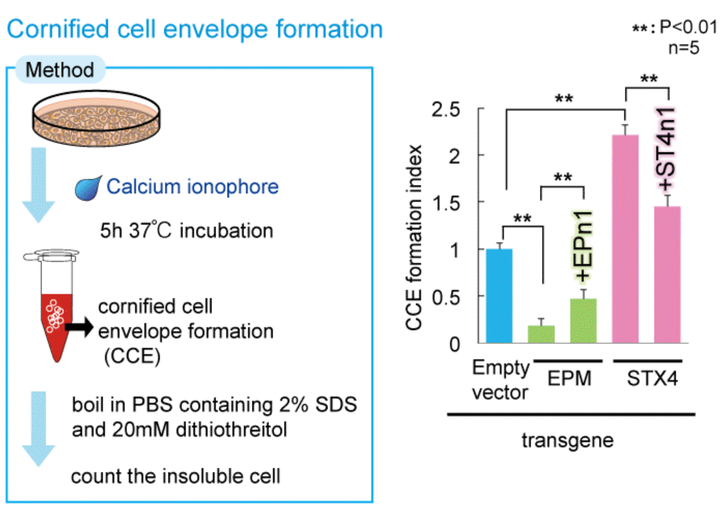
Preparation of a possible antagonistic peptide of STX4, based on the generation process of EPn1 from EPM.

antagonistic action of ST4n1

competitive inhibition?

6. Effect of STX4 and ST4p

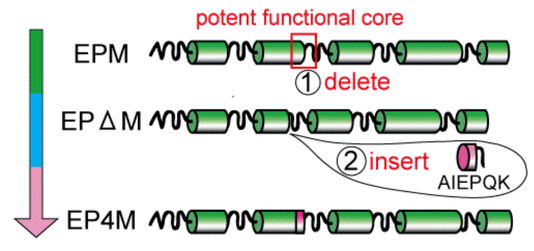
Cornified cell envelope formation



The CCE formation was suppressed by extracellular EPM, but enhanced by that of STX4, each of which was neutralized by EPn1 or ST4n1, respectively.

7. Generation of EPΔM,EP4M

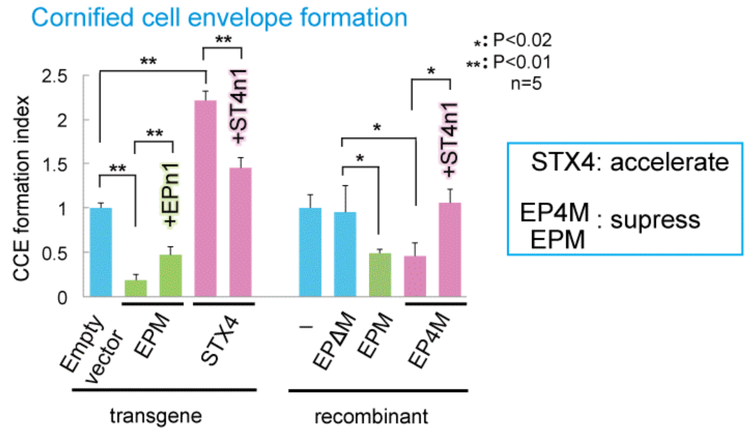
EPM mutants focusing on
"the predicted potent functional core of STX4"



① deletion of the potent functional core from EPM
② insertion of the corresponding peptide from STX4 to ①

8. Effects of EPΔM,EP4M

Cornified cell envelope formation

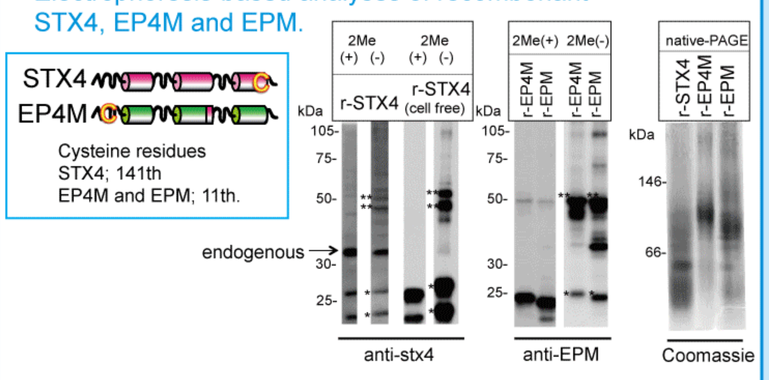


STX4: accelerate
EP4M : supress EPM

EP4M appeared to behave like EPM, which was opposite to STX4. ST4n1 blocked both of these conflicting effects.

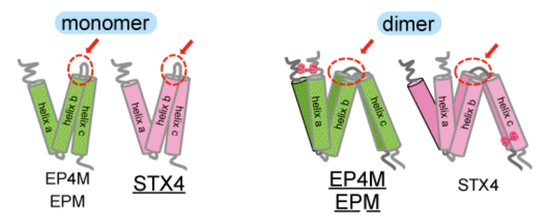
9. Electrophoresis-based analyses

Electrophoresis-based analyses of recombonant STX4, EP4M and EPM.



The majority of r-stx4 existed as a monomer (*) (left panel), whereas r-EPM or r-EP4M formed dimers (**) (middle panel). Native-PAGE electrophoresis demonstrated that STX4 ran much faster than EPM and EP4M (right panel).

Expected model



STX4, EP4M and EPM form dimers. Oligomer formation leads to the structural alternation of the functional core?

10. Conclusion

- STX4 led to an acceleration of CCE formation. EP4M did not behave like STX4 but rather like EPM in terms of a suppression of CCE formation.
- A newly generated circular peptide from the potent functional core of STX4 (ST4n1) clearly neutralized cellular responses elicited by STX4 and EP4M.
- EP4M and EPM mainly existed as dimers/oligomers, whereas STX4 mainly remained as monomer.

11. Discussion

- STX4 might lead to the hyperplasia of horny layer.
- ST4n1 could be utilized to remedy epidermal abnormal behaviors caused by STX4.