研究例 4

細胞の癌化･転移機構の解析

Sustained expression of LIP, a short repressive isoform of C/EBPβ, leads to epithelial-mesenchymal transition (EMT) in the mammary epithelial cells

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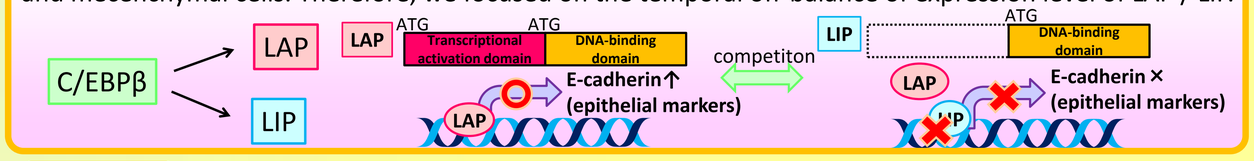
1. Background

Upon oncogenic transformation, epithelial cells temporally display a mesenchymal phenotype (EMT), in which a transcription factor Snail1 plays a causal role for various epithelia. Recently, another transcription factor CCAAT/enhancer binding protein C/EBPβ has been implicated to elicit the similar response in mammary epithelial cells. C/EBPβ mRNA has two translation initiation sites in its open reading flame, thereby is translated into two isoforms, LAP (liver-enriched activating protein) that is composed of the transcription and DNA binding domains, and LIP (liver-enriched inhibitory protein) that has solely DNA-binding domain. LIP is known as a competitor of LAP, and it cannot activate transcription of LAP targets. However, no difference between the amount of LAP protein and LIP protein was observed in epithelial and mecenchymal cells. Therefore, we focused on the temporal off-balance of expression level of LAP / LIP.

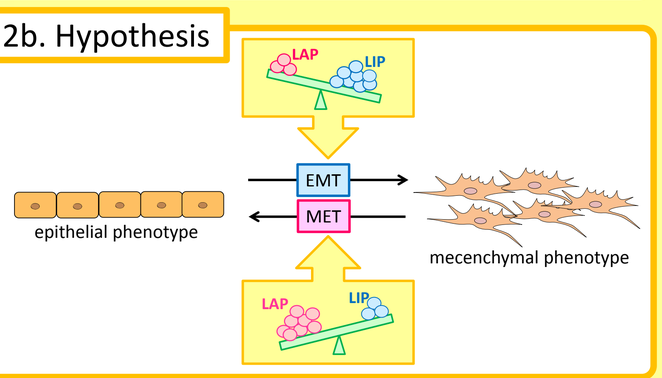


2a. Aim

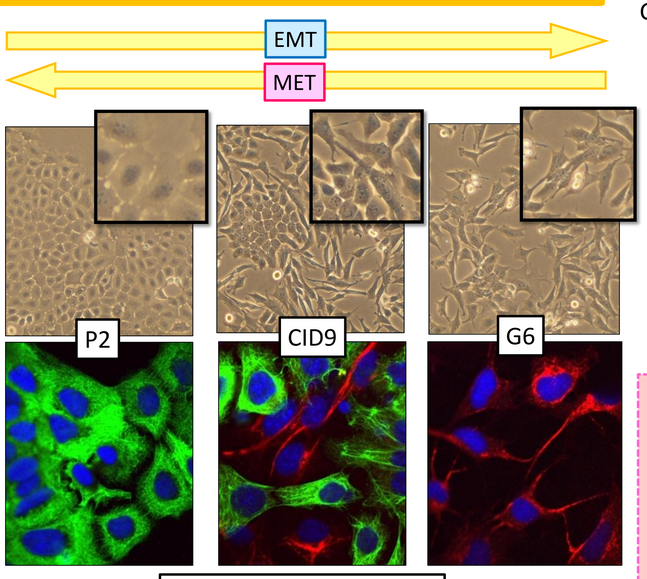
Elucidation of the possible involvement of LAP / LIP in epithelial-mecenchymal transition (EMT) and mecenchymal-epithelial transition (MET).

- Forced expression of LIP in epithelial cells leads to EMT?
- Forced expression of LAP in mecenchymal cells leads to MET?

2b. Hypothesis



3. Model cells and markers for EMT / MET



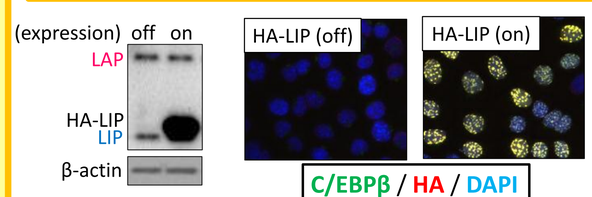
COMMA1-D (mammary epithelial cells)

- P2 : pure epithelial population
- G6 : transformed population (fibroblastic)

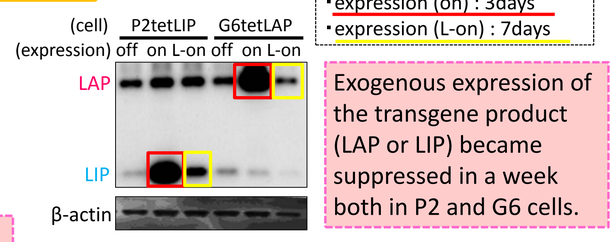
	P2	CID9	G6
cell	epithelial	epithelial / mecenchymal	mecenchymal
keratin	◎	○	×
E-cadherin	◎	○	×
vimentin	×	○	◎

- P2 cells express epithelial markers, keratin and E-cadherin.
- G6 cells express a mecenchymal marker, vimentin.
- CID9 cells express both epithelial and mecenchymal markers.
- EMT-transitioning cells are resemble to CID9 cells.
- P-cadherin, MMP2, MMP3 are other EMT markers.

4. Generation of inducible cells (tet-off system)



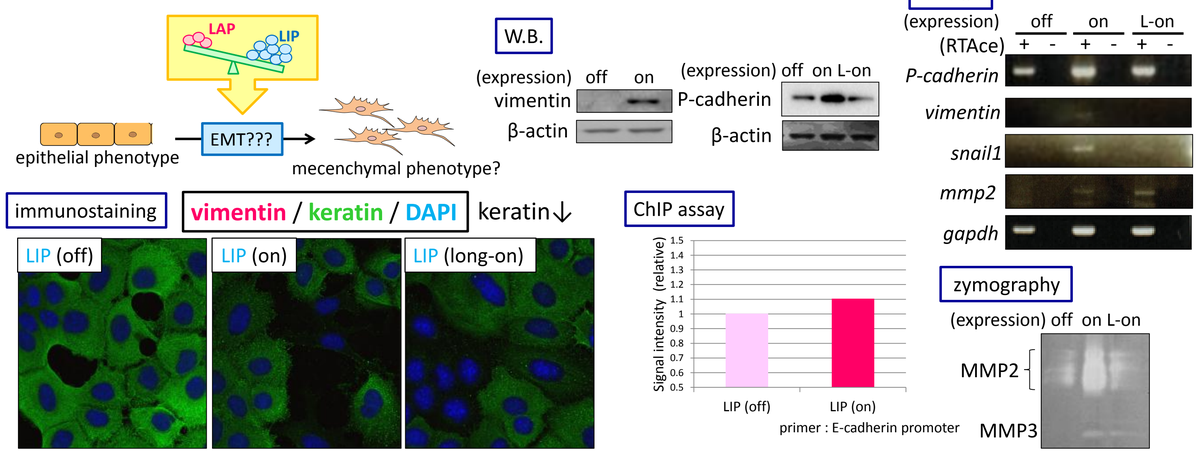
Exogenous LIP was exclusively expressed in cell nuclei.



- expression (on) : 3days
- expression (L-on) : 7days

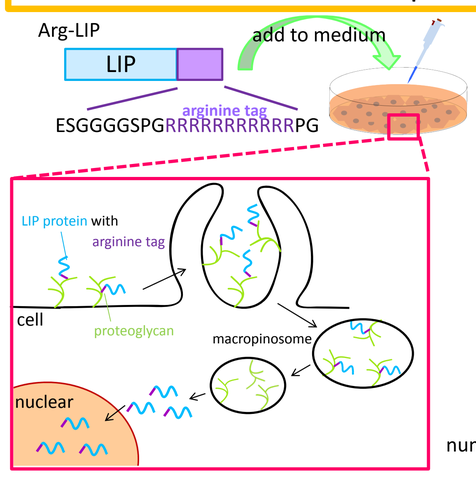
Exogenous expression of the transgene product (LAP or LIP) became suppressed in a week both in P2 and G6 cells.

5. Effects of exogenous LIP on P2 cells

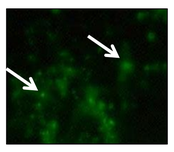


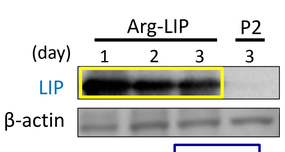
- Forced expression of LIP protein temporally induced EMT-like cell behaviors.
- The expression profile of the EMT-markers was consistent with that of exogenous LIP protein.

6. Direct introduction of LIP protein into P2 cells

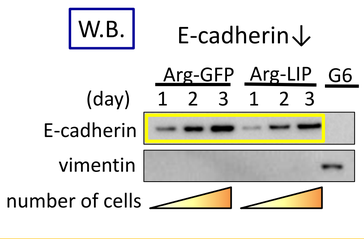


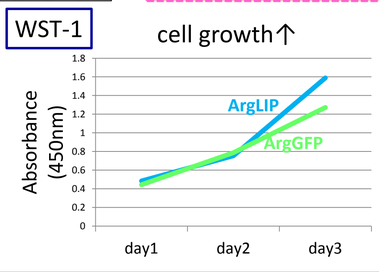
Recombinant LIP / GFP protein added to medium was successfully delivered to nuclei.



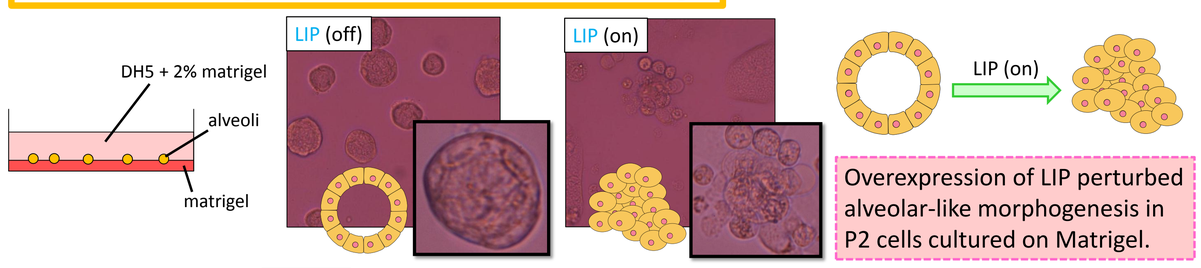


Again, LIP protein transported to cell nuclei elicited EMT-like changes in P2 cells.

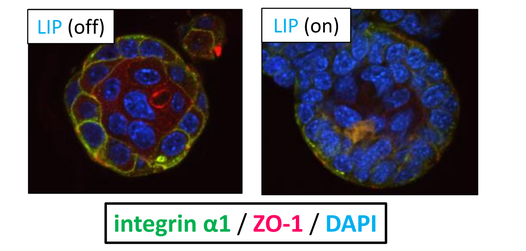


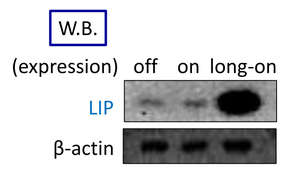


7. Effects of exogenous LIP on P2 cells on matrigel



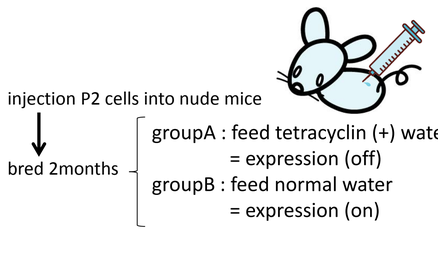
Overexpression of LIP perturbed alveolar-like morphogenesis in P2 cells cultured on Matrigel.

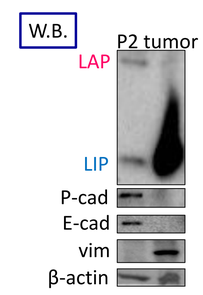




In contrast to on plastic, P2 cells cultured in matrigel maintain LIP-overexpression.

8. Effects of exogenous LIP on P2 cells *in vivo*

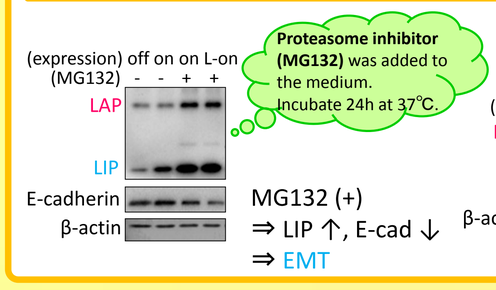




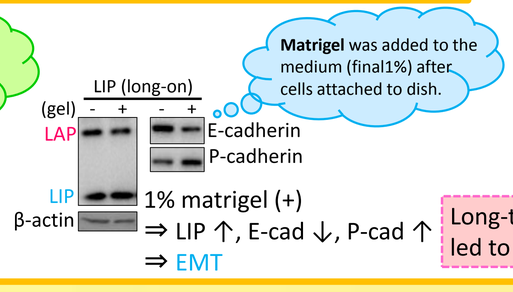
	A1	A2	A3	A4	B1	B2	B3
LIP expression	off	off	off	off	on	on	on
palpable cancers	×	×	×	×	◎	◎	◎
weight (g)	19.5	20.7	21.0	19.0	23.6	24.9	24.0
tumors (g)	0	0	0	0	0.8	0.5	2.0
spleen (g)	0.1	0.1	0.1	0.1	0.4	0.3	0.7

In vivo, P2 cells with overexpression of LIP formed metastatic tumors. The expression of LIP was sustained for a long period, which was accompanied with a dramatic alternation in the expression of EMT-markers in the tumors.

9. Proteasome-dependent elimination of excess amount of LIP



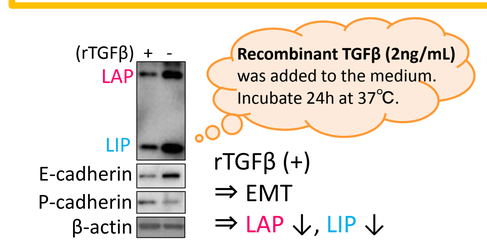
MG132 (+) ⇒ LIP ↑, E-cad ↓ ⇒ EMT



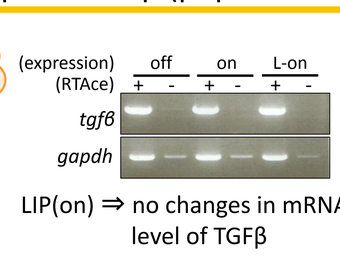
1% matrigel (+) ⇒ LIP ↑, E-cad ↓, P-cad ↑ ⇒ EMT

Long-term expression of LIP led to clearer EMT in P2 cells.

10. Relationship between C/EBPβ and TGFβ (popular EMT inducer)



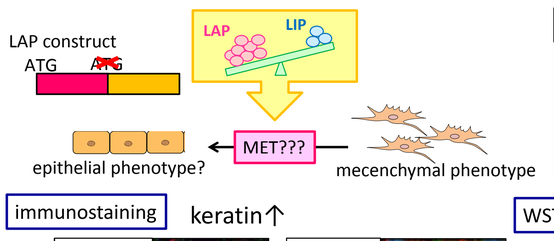
rTGFβ (+) ⇒ EMT ⇒ LAP ↓, LIP ↓

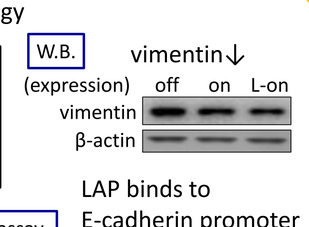


LIP(on) ⇒ no changes in mRNA level of TGFβ

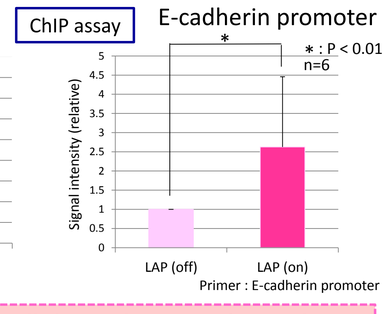
C/EBPβ is a downstream of TGFβ signaling pathway.

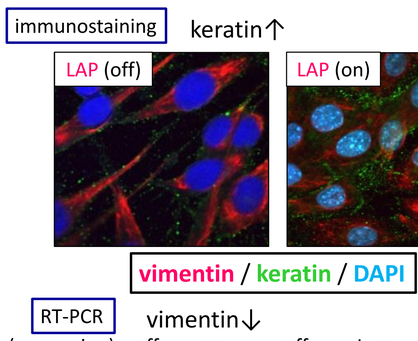
11. Effects of exogenous LAP on G6 cells

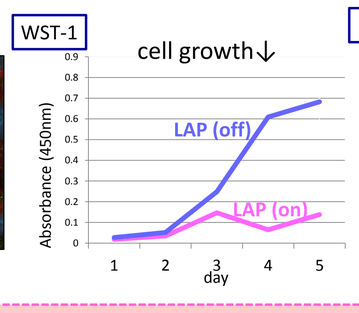


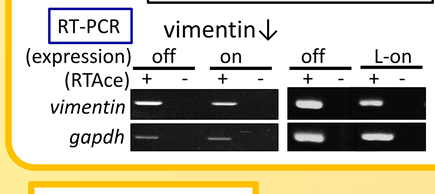


LAP binds to E-cadherin promoter









Overexpression of LAP resulted in the cell shape change and growth arrest in G6 cells. LAP also led to the down-regulation of a mecenchymal marker and the up-regulation of an epithelial marker. LAP elicited cellular response opposite to LIP.⇒ LAP might induce MET.

12. Conclusion

- LIP induced EMT-like changes and abrogated morphogenic potential in mammary epithelial cells.
  LIP overexpression in the epithelial cells resulted in the formation of metastatic tumors.
- LAP induced MET-like changes in mecenchymal cells.

- The expression profile of LIP protein was affected by cellular microenvironment.
  ⇒Excess amount of LIP protein was actively eliminated.
  ⇒Under the influence of basement membrane, the LIP-elimination was suppressed.
- Possibility : LIP also acts as a transcriptional activator in mammary.
- C/EBPβ is a downstream of TGFβ signaling pathway.