Nov. 13, 2015
JSSX Award Lecture

Transport and toxicity of the drug and its regulation in the kidney and lung

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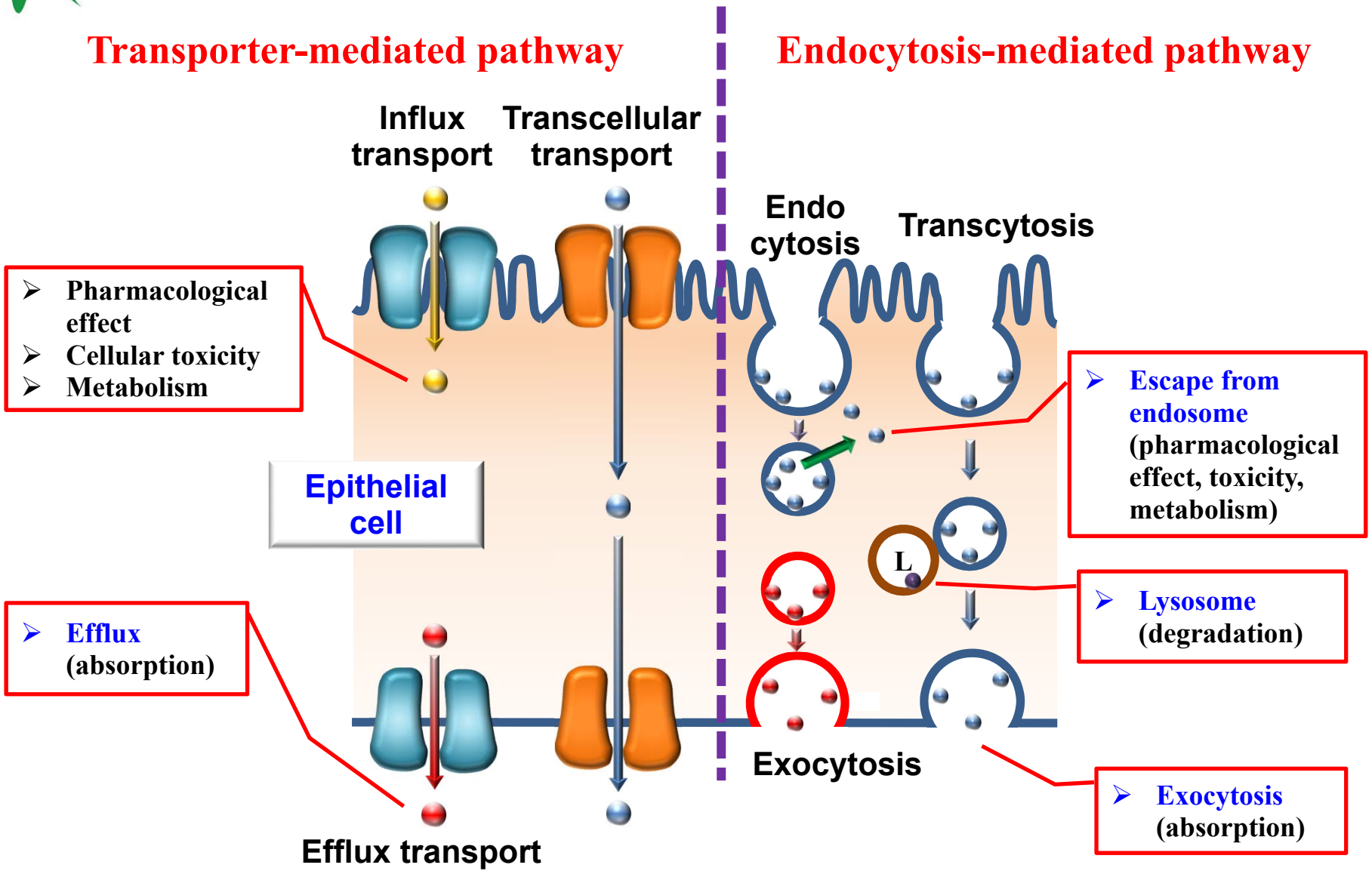
Role of membrane transport and endocytosis processes in the pharmacokinetics, efficacy, and toxicity of drugs



Topics of today's talk

《Kidney》

1-1) Molecular aspects of renal handling of aminoglycosides

1-2) Strategy for preventing aminoglycoside-induced nephrotoxicity

《Lung》

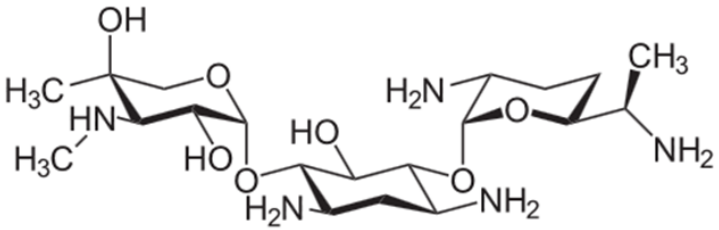
2-1) Mechanism of protein (albumin) transport in lung alveolar epithelial cells

2-2) Strategy for enhancing the clearance/absorption of albumin from the lung

3) Epithelial-mesenchymal transition as a cause of drug-induced lung toxicity

Aminoglycosides (AGs) and their renal toxicity

Gentamicin (GM)



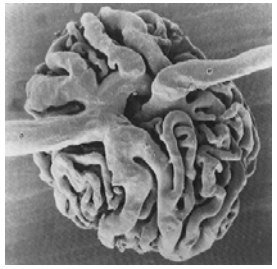
Characteristics of AGs

- Antibiotics widely used for Gram-negative infectious diseases
- Water soluble polycation

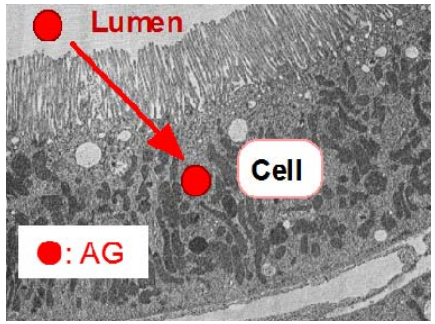
《PK》

- Plasma protein binding: low (< 10 %)
- Main elimination pathway: renal excretion

Glomerulus



Proximal tubular epithelial cells

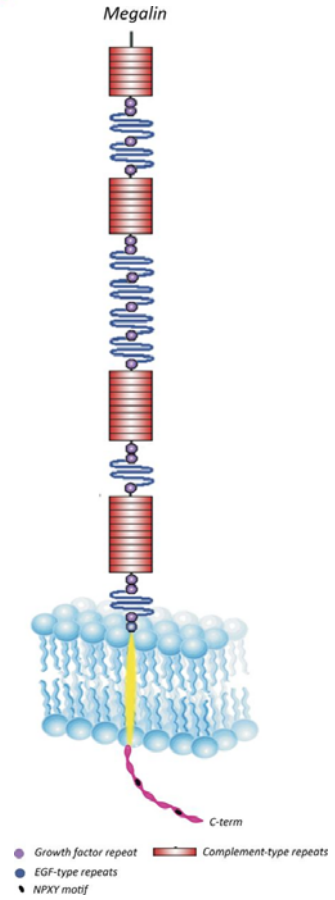


Renal Handling of AGs

Administration
↓
Freely filtered through the glomerulus
↓
Excreted into the urine (> 90% of injected dose)
↓
Selectively accumulated in renal proximal tubules (5-10%)
↓
Nephrotoxicity

Using kidney epithelial cell line, I previously reported that gentamicin was taken up by receptor-mediated endocytosis (J Pharmacol Exp Ther, 1994). However, the receptor responsible for aminoglycoside uptake was unknown.

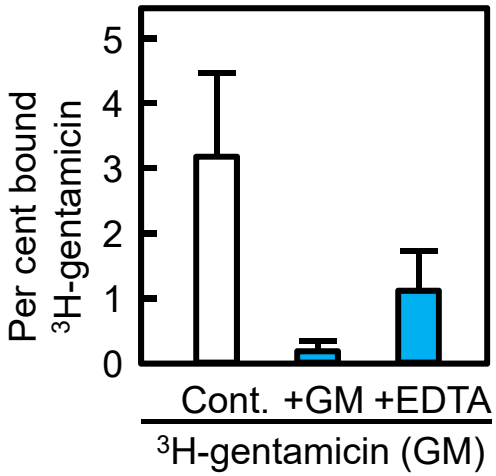
Structure and function of megalin and in-vitro interaction of AG with megalin



American Journal of Physiology - Renal Physiology, 306, F147, 2014

Megalin

- Large membrane protein: about 600 kDa (mega-)
- Endocytosis receptor
- Ligands of megalin: vitamin D binding protein, receptor-associated protein (RAP), cytochrome c, etc.
- Alias: Heymann nephritis autoantigen, LDL-receptor-related protein-2 (LRP-2; LDL receptor family)



GM can bind to "Megalin" (in-vitro)

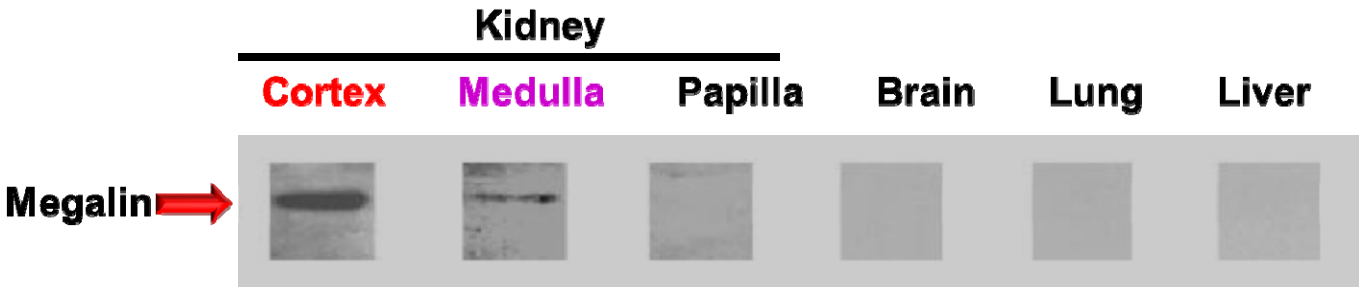
Moestrup SK et al., J Clin Invest 1995;96:1404-13

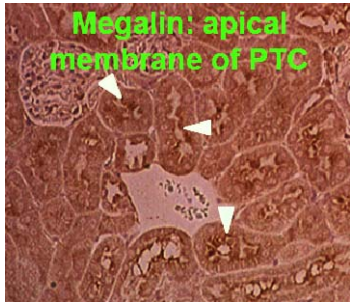
Key Question: Is megalin the receptor for aminoglycoside uptake in the kidney?

Role of megalin in renal accumulation of AGs

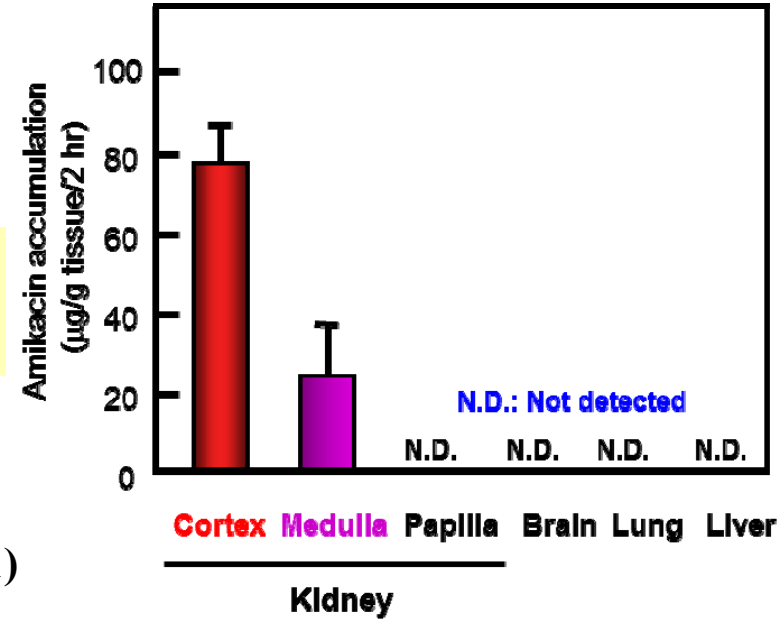
Tissue distribution: megalin expression and amikacin accumulation

Western blot analysis of rat tissue homogenates with anti-megalin antiserum against intracellular domain of megalin





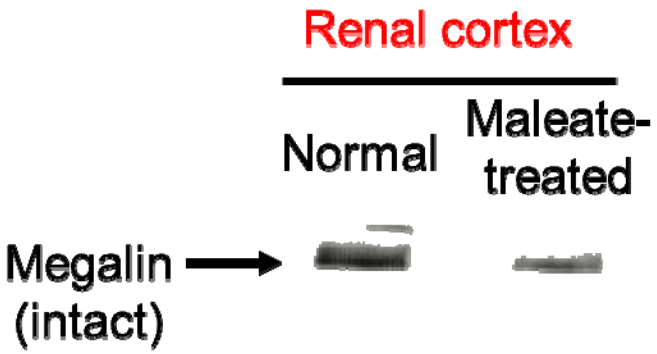
Tissue distribution pattern of amikacin after iv injection

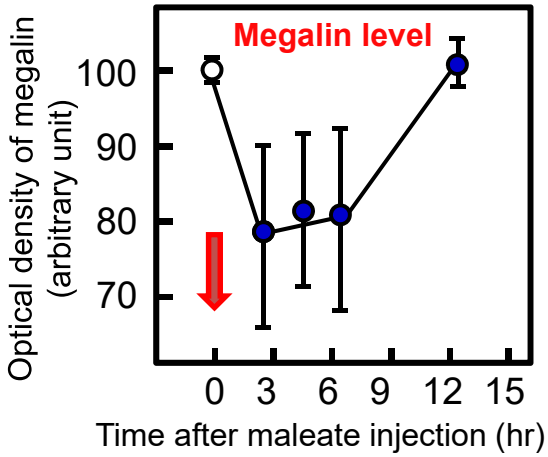


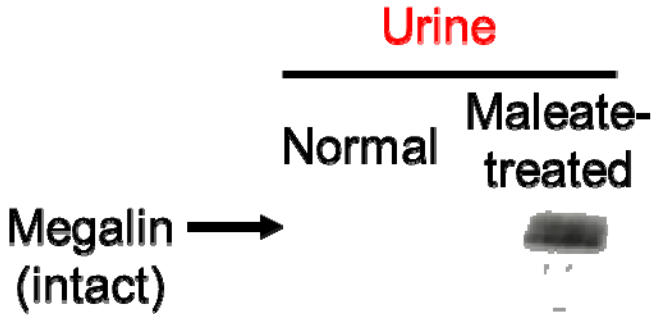
Am. J. Physiol., 281 (2): F337-F344 (2001)

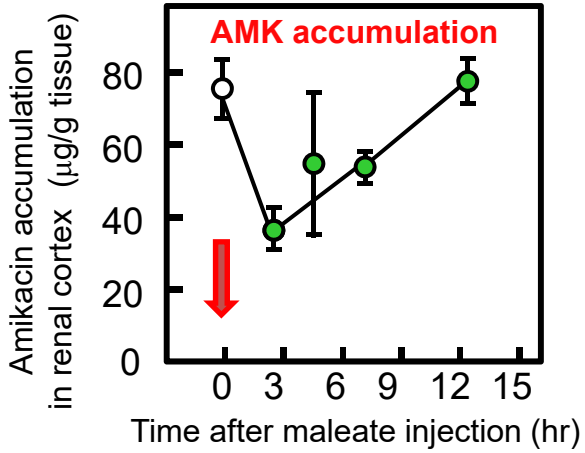
Role of megalin in renal accumulation of AGs

Relationship between renal megalin level and amikacin accumulation after maleate treatment in rats



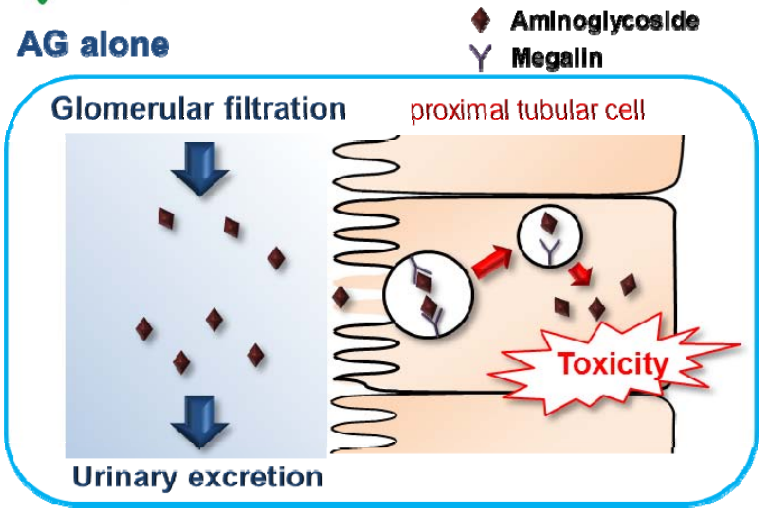


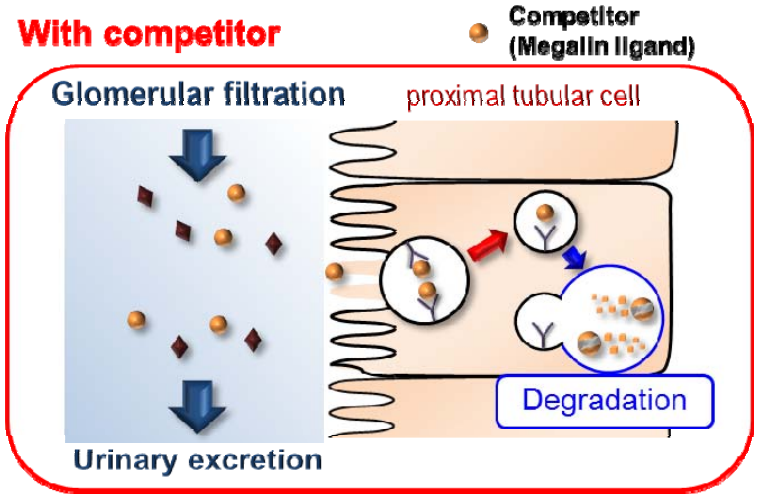




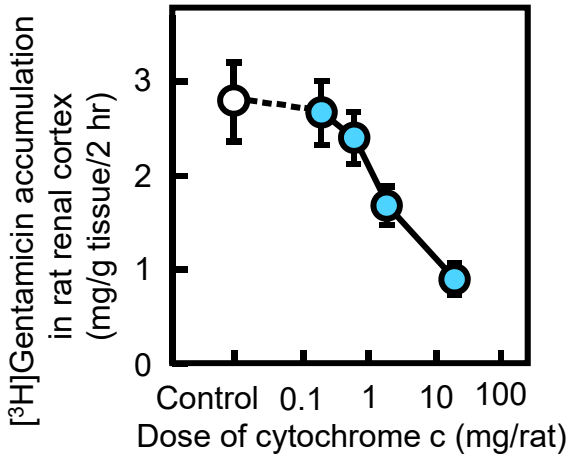
Megalin is the receptor responsible for AG accumulation in the kidney under in-vivo condition.

Strategy to prevent renal accumulation and toxicity of AGs

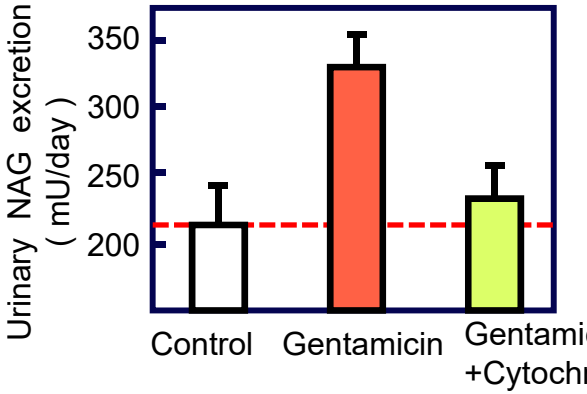




Effect of co-administration of cytochrome c on [^{3}H]gentamicin accumulation in renal cortex in rats



Effect of cytochrome c on renal toxicity of gentamicin estimated by urinary excretion of N-acetyl-β-D-glucosaminidase (NAG) in rats



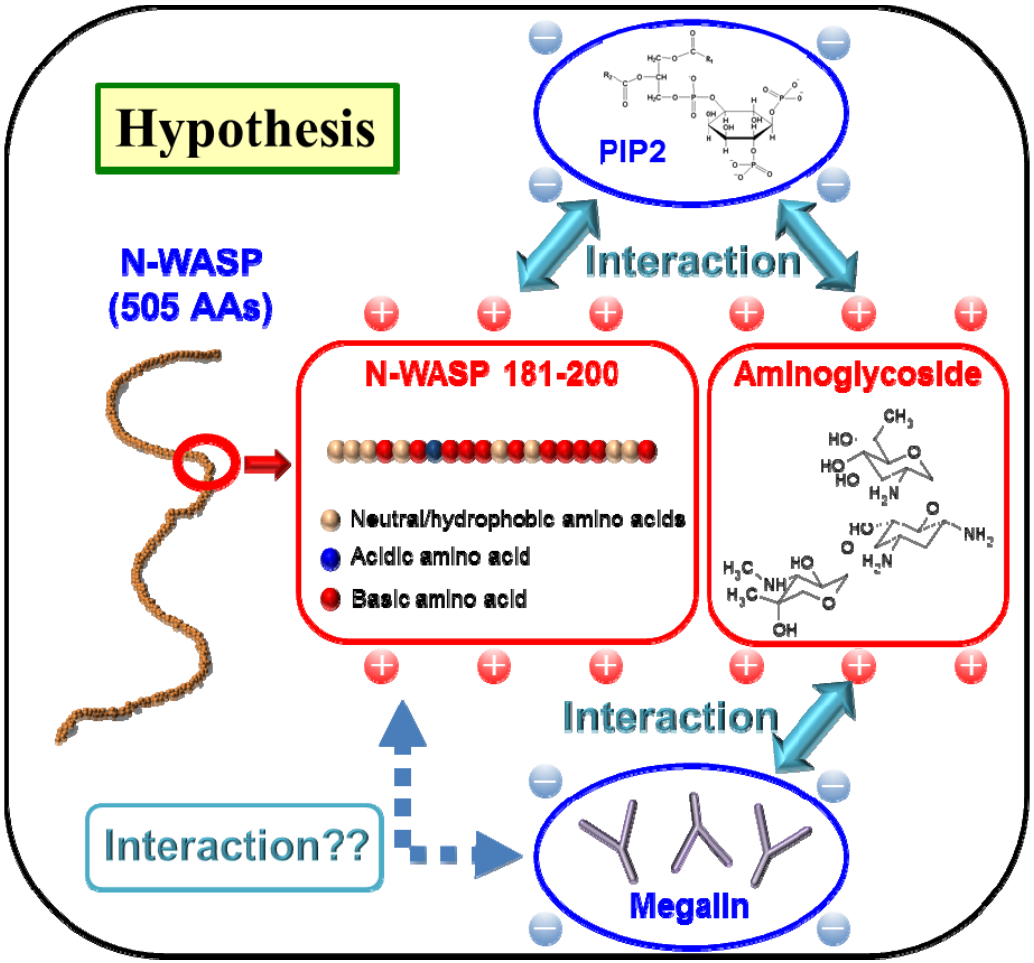
Expert Opin. Drug Metab. Toxicol. (2010)

Search for smaller peptides which can inhibit megalin-mediated endocytosis of AGs

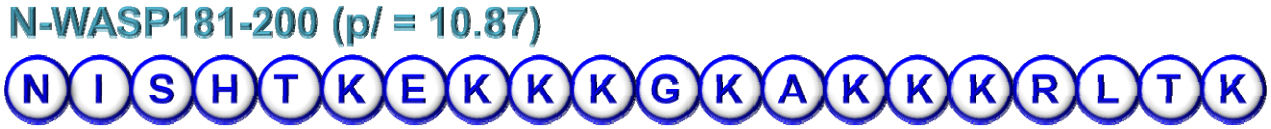
N-WASP and N-WASP181-200

- Neural Wiskott-Aldrich syndrome protein (N-WASP) is a protein which regulates actin polymerization.
- PI (4,5)P_2 (Phosphatidyl inositol 4,5-bisphosphate) binds to the basic motif in N-WASP (N-WASP181-200; calculated p*I* = 10.87) and activates N-WASP function.

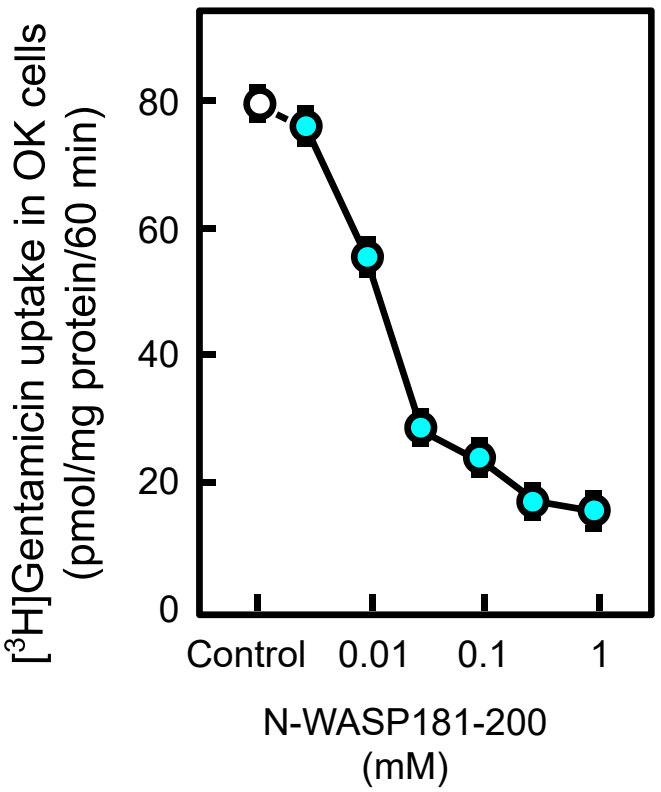
Toshiki Itoh, Regulation of actin cytoskeleton by phosphoinositides, MEMBRANE (2002)



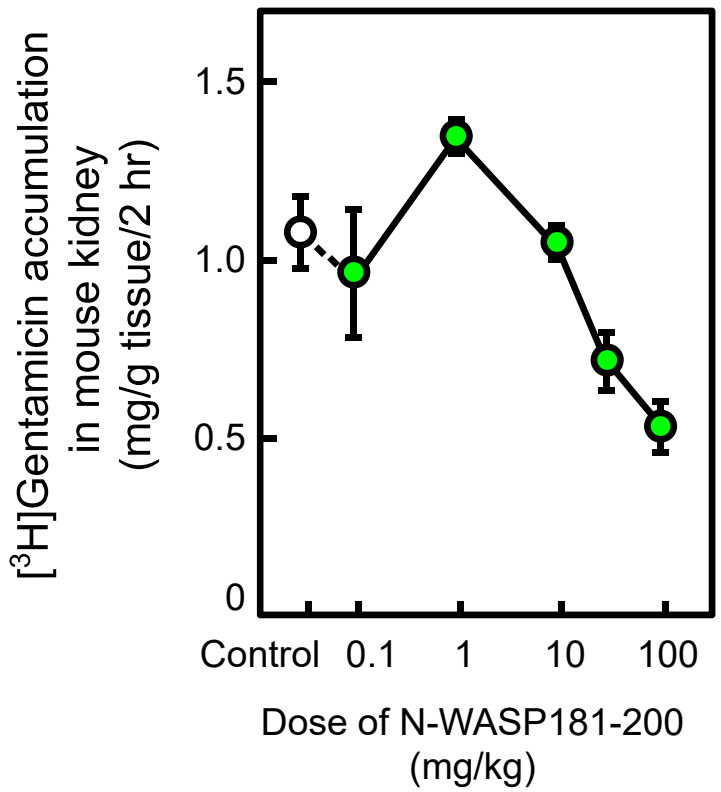
Effect of N-WASP181-200 on gentamicin accumulation in renal epithelial cells



Uptake in OK cells
(In vitro)



Renal accumulation in mice
(In vivo)



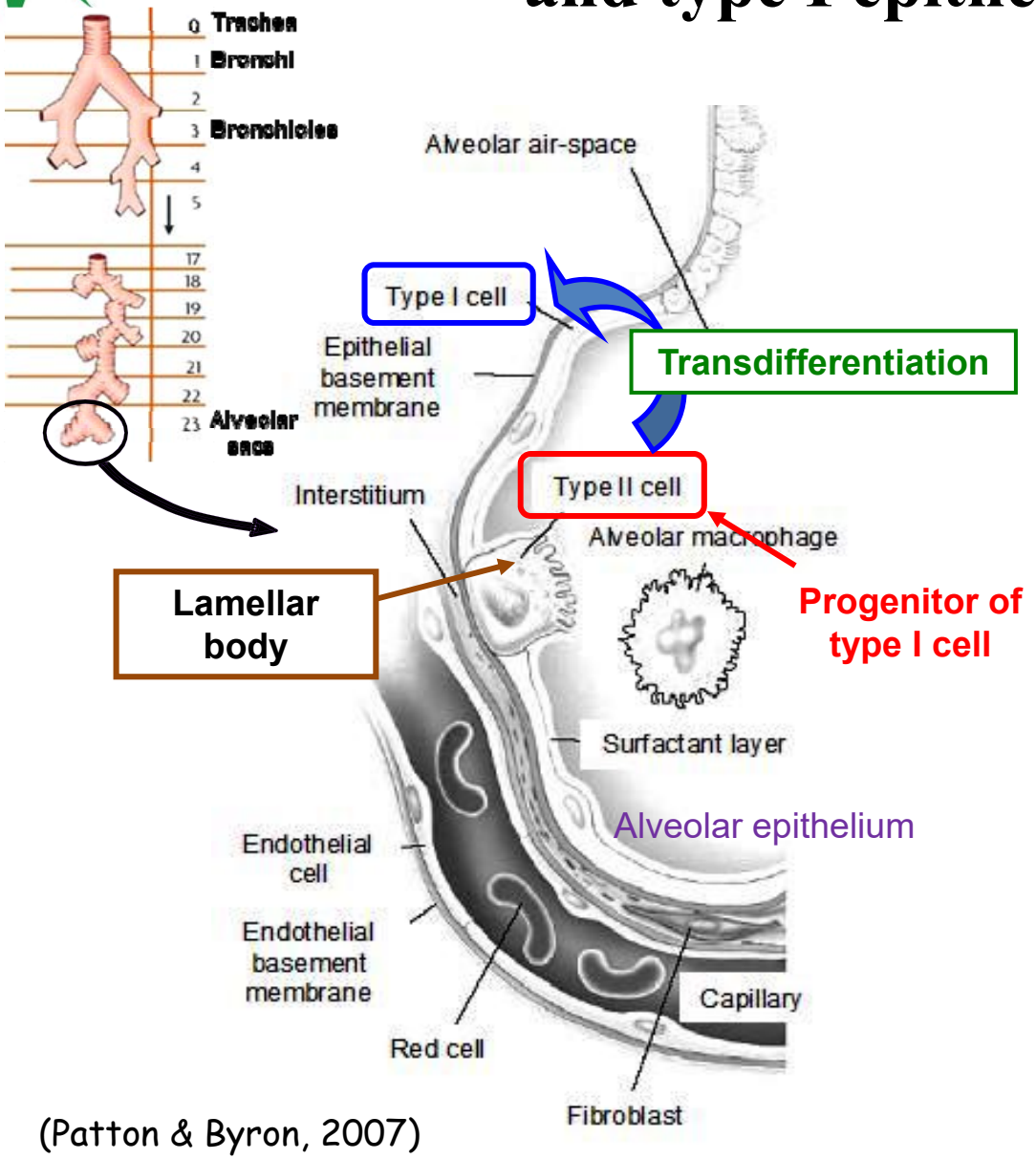
J. Contl. Rel., 95 423-433 (2004)

Summary (1)

- Megalin is the receptor responsible for the endocytosis of aminoglycoside into the renal proximal tubular cells under in vivo condition.

- Megalin ligands can inhibit the renal accumulation of aminoglycoside and its nephrotoxicity. Cationic peptides such as N-WASP 181-200 and its derivatives may be good candidates as the megalin competitor to prevent aminoglycoside nephrotoxicity.

Structure and function of lung alveolus and type II and type I epithelial cells



(Patton & Byron, 2007)

There are about 400-500 million alveoli in the distal lung, and the total surface area is > 100 m^2.

Type I cells:

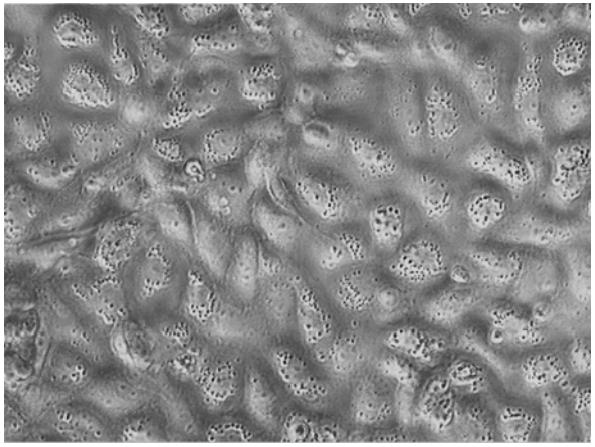
- squamous and thin epithelial cells
- 90-95% of surface area
- gas exchange

Type II cells:

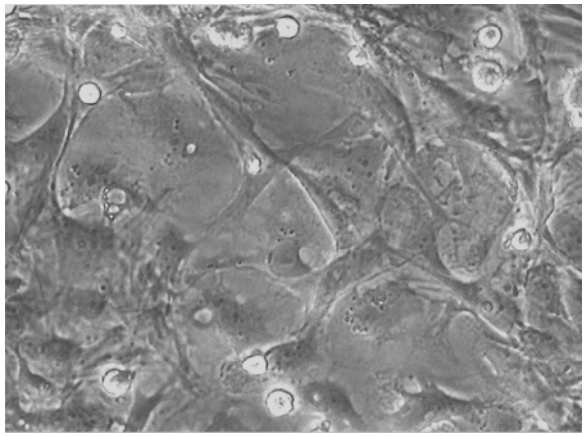
- cuboidal epithelial cells
- 5-10% of surface area
- surfactant production

Morphology of primary cultured alveolar type II and type I-like epithelial cells

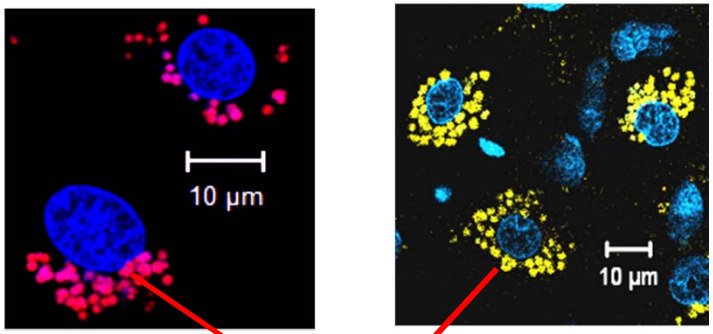
Type II cells
(5 x 10^6 cells/35 mm dish/2 days)



Type I-like cells
(2 x 10^6 cells/35 mm dish/6 days)



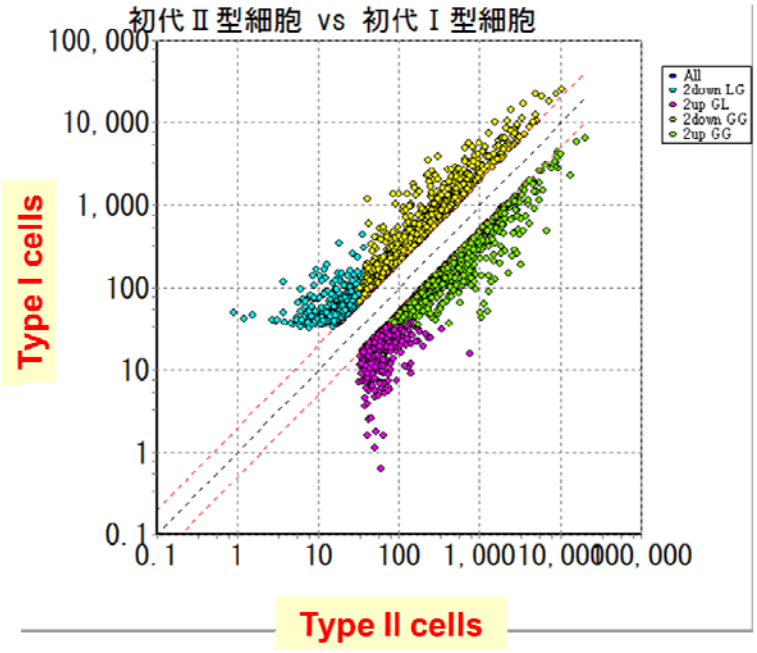
Confocal laser scanning micrographs of lamellar bodies in type II cells



LB: pulmonary surfactant production

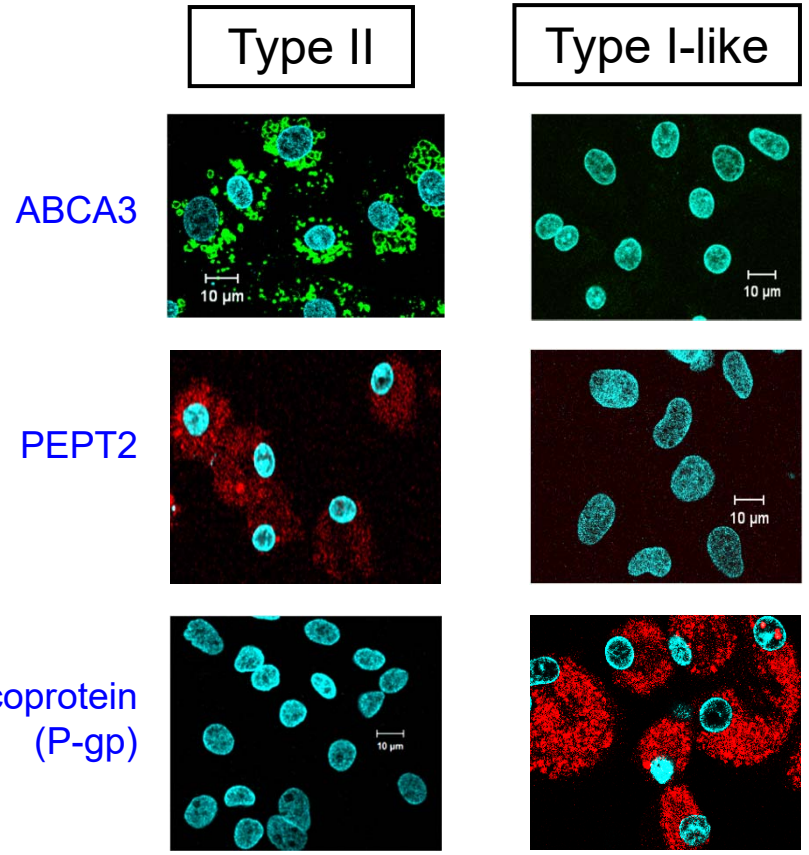
Change in mRNA and protein expression profiles along with transdifferentiation

mRNA expression profile (Microarray analysis)



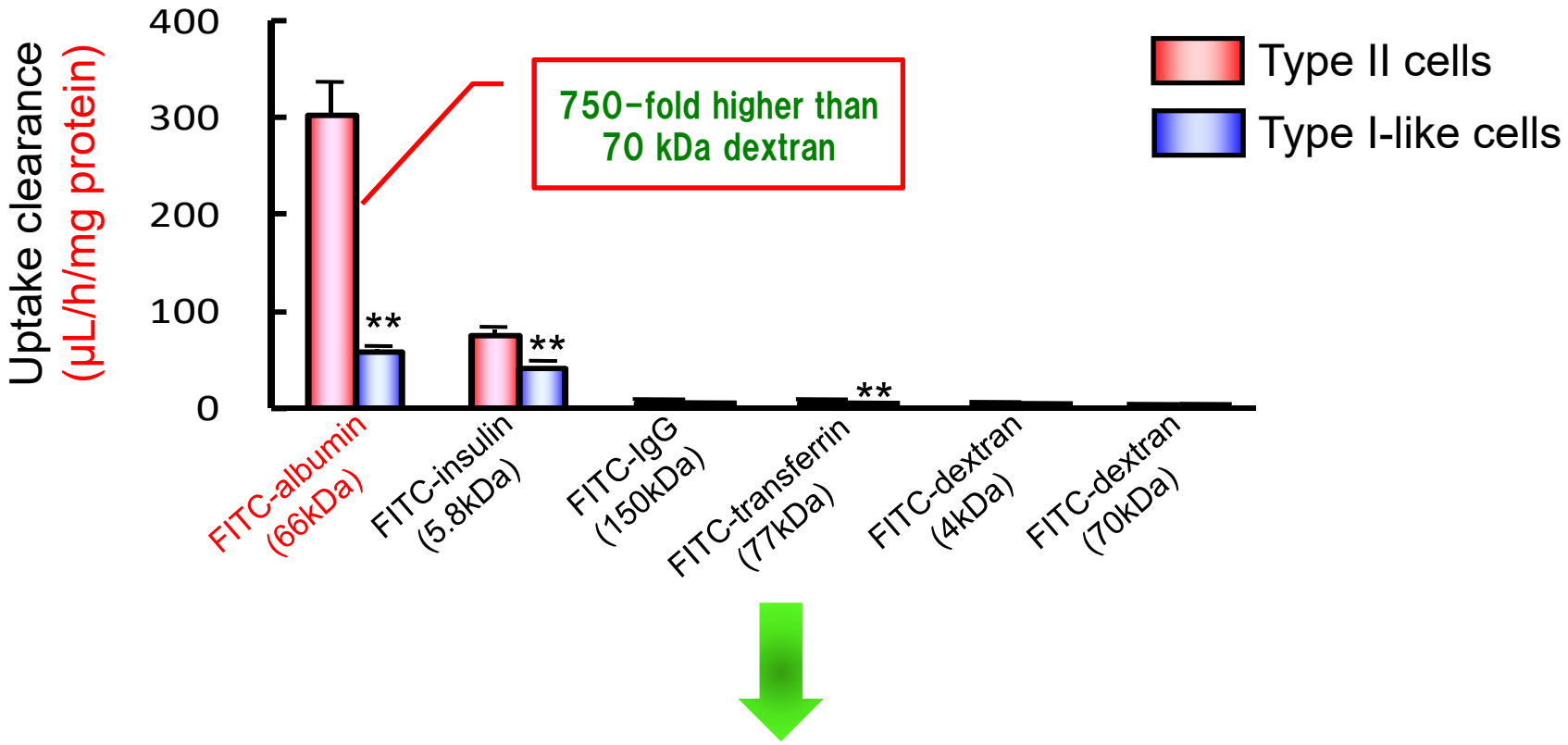
> 2.0-fold (about 1200/35000 mRNAs)
< 0.5-fold (about 1200/35000 mRNAs)

Protein expression profile (Immunostaining)



Life Sciences 93, 630-636 (2013)

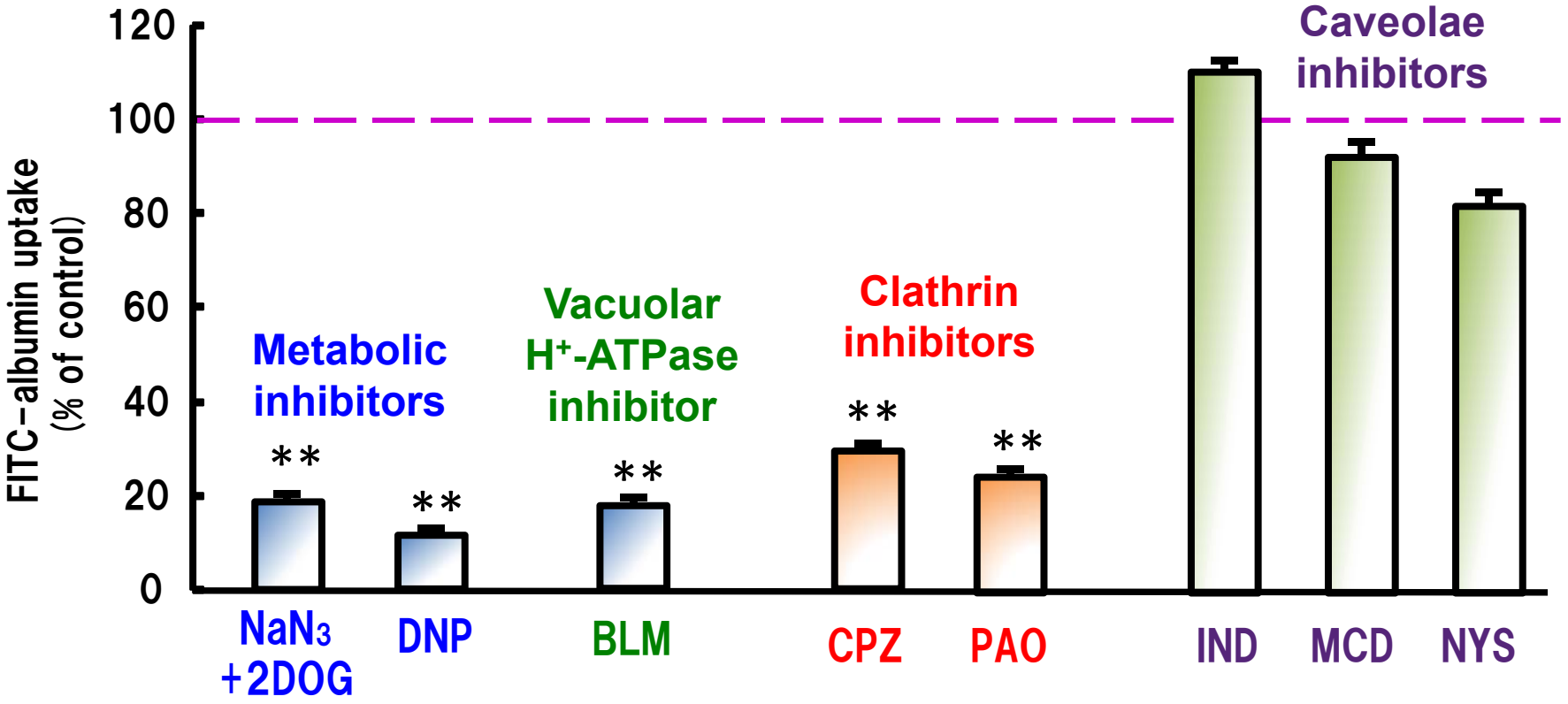
Transport of albumin in primary cultured alveolar type II and type I-like epithelial cells



Based on albumin uptake clearance per one cell and the ratio of type II and type I cell number in the lung (type II > type I), the contribution of type II cells for the total albumin clearance from the alveolar lining fluid was estimated to be more than 70%.

Pharm. Res., 25, 913-922 (2008)

Mechanism and pathway of albumin uptake in type II cells: Clathrin-mediated endocytosis



Sodium azide: NaN_3
2DOG: 2-deoxy-D-glucose
DNP: 2,4-dinitrophenol
BLM: Bafilomycin A_1

CPZ: chlorpromazine
PAO: phenylarsine oxide

IND: indomethacin
MCD: methyl-β-cyclodextrin
NYS: nystatin

**p<0.01 *vs.* each control.

Role of albumin clearance from alveolar lining fluid under pathophysiological conditions

- The concentration of albumin in alveolar fluid is normally about 10%, but increases to 75-95%, of the plasma level in lung injury pulmonary edema.
- Alveolar clearance of protein is assumed to be a critical process in fluid clearance and therefore recovery from the edema.

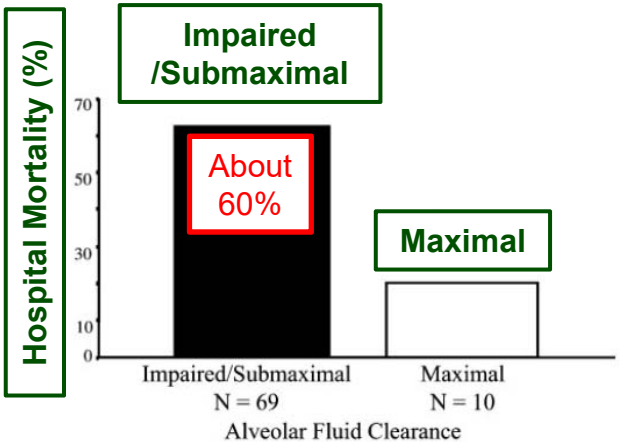


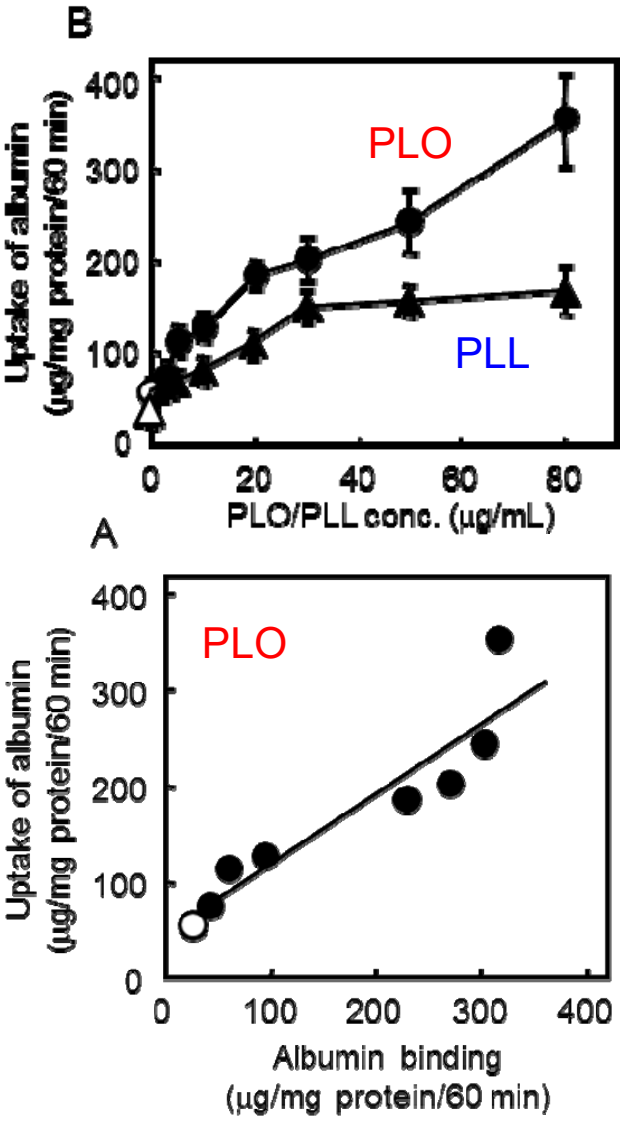
FIG. 6. Hospital mortality (*y*-axis) plotted against two groups of patients with acute lung injury or the acute respiratory distress syndrome: those with maximal fluid clearance (>14%/h) and those with impaired or submaximal fluid clearance (<14%/h). The columns represent percent hospital mortality in each group (*n* = number of patients). Hospital mortality of patients with maximal fluid clearance was significantly less ($P < 0.02$). [From Ware and Matthay (375), with permission from The American Thoracic Society.]

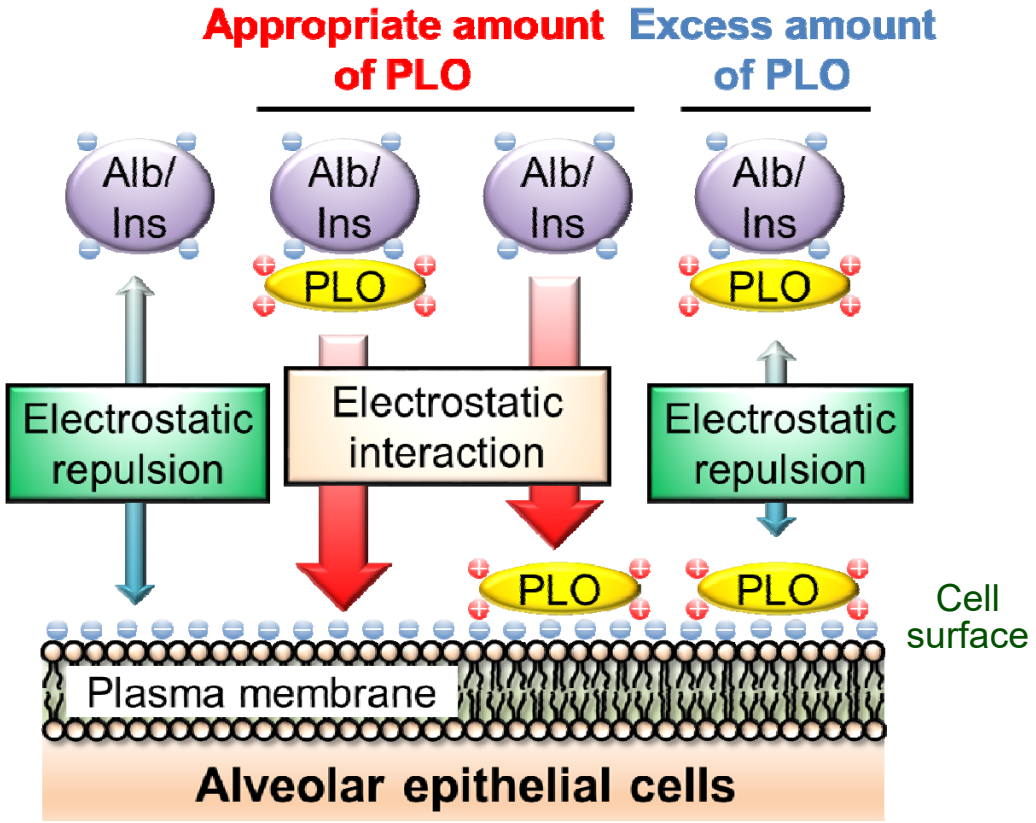
Clinically, the hospital mortality was high in lung injury patients with impaired/submaximal fluid clearance than that with maximal fluid clearance.

Development of a strategy to enhance the albumin clearance would be helpful for the early recovery from the lung injury accompanied with edema.

Pulmonary administration of a compound that can enhance albumin clearance would be a simple but a promising strategy.

In-vitro effect of cationic poly(amino acid)s on albumin uptake in alveolar epithelial cells (A549)



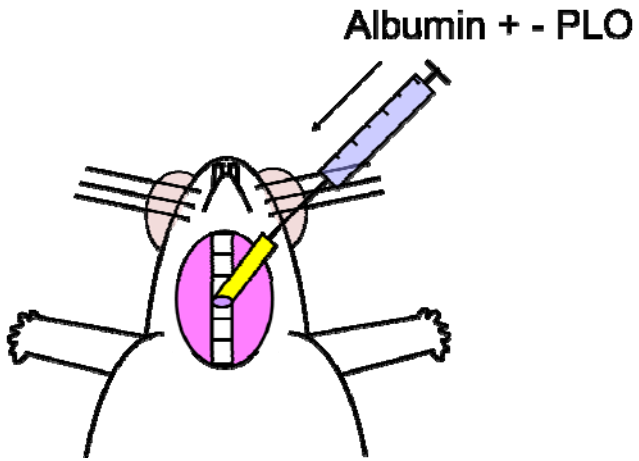


PLO: poly-L-ornithine PLL: poly-L lysine

Expert Opin. Drug Delivery (2015)

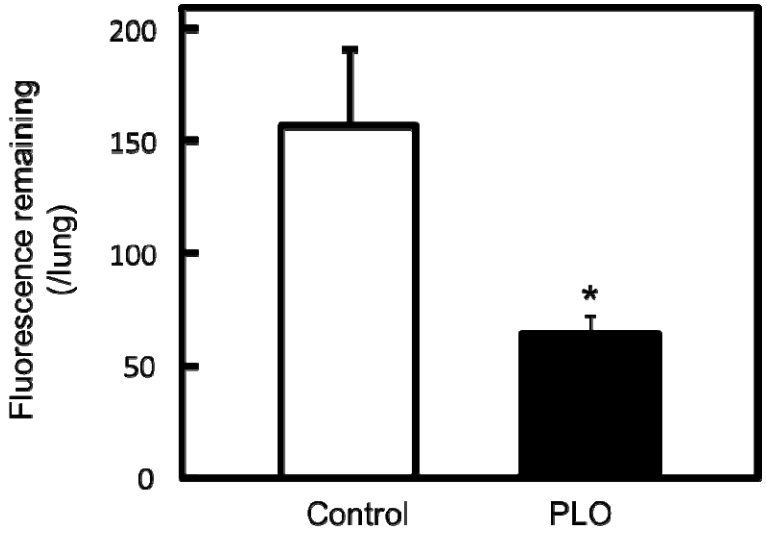
In-vivo effect of PLO on albumin clearance from the lung

In vivo pulmonary administration experiments

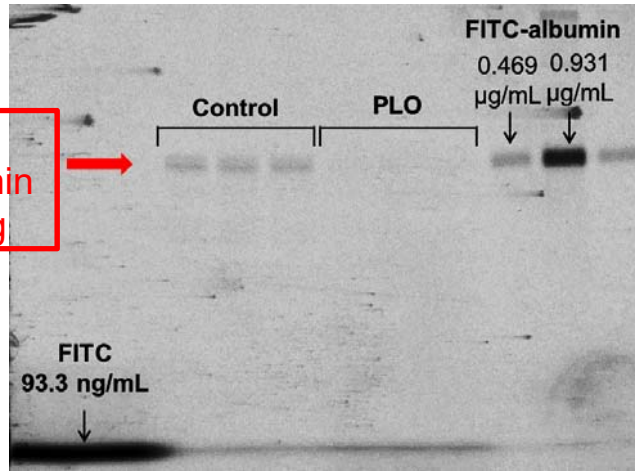


Animal: Male Wistar rats
Albumin conc.: 200 μg/mL
PLO conc.: 240 μg/mL
Volume: 50 μL
Time: 2 hours after administration

Fluorescence derived from FITC-albumin



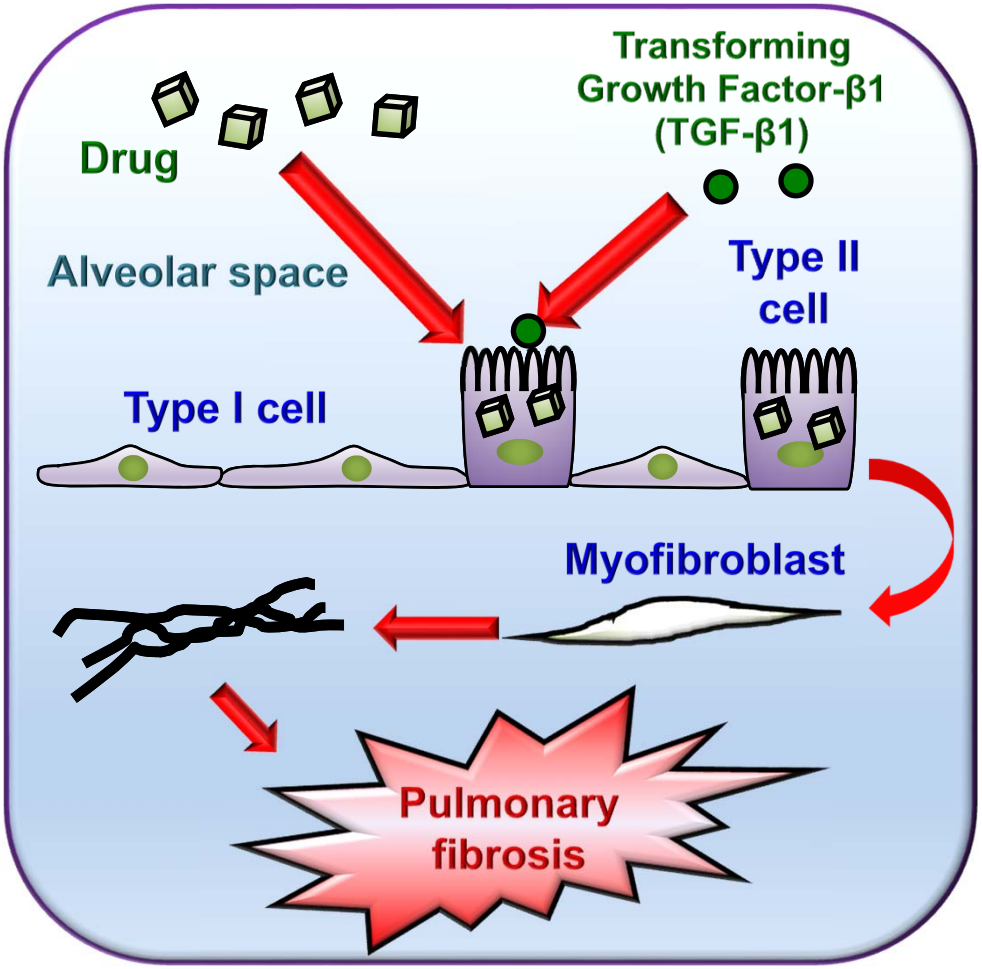
Intact FITC-albumin in the lung



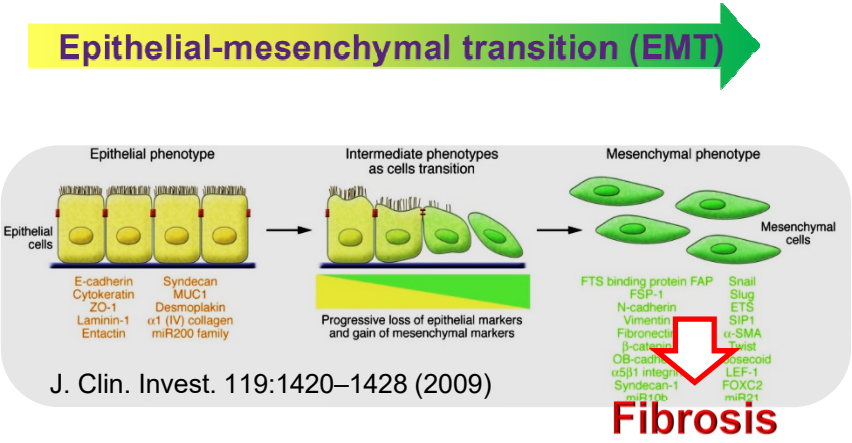
Summary (2)

- Though the surface area occupied by type II cells is much smaller (5-10%) than that by type I cells, type II cells would significantly contribute to albumin uptake in the lung.
- Albumin is endocytosed into type II cells via a clathrin-mediated pathway, but not via a caveolae-mediated pathway.
- Cationic poly(amino acid)s such as poly-L-ornithine (PLO) stimulated albumin endocytosis into the alveolar epithelial cells and enhanced the in-vivo clearance of albumin from the lung. Therefore, pulmonary administration of PLO may be a possible strategy for facilitating the recovery from pulmonary edema.
- PLO was also useful for enhancing insulin absorption from the lung, and potentiated the pharmacological effect of insulin (data not shown).

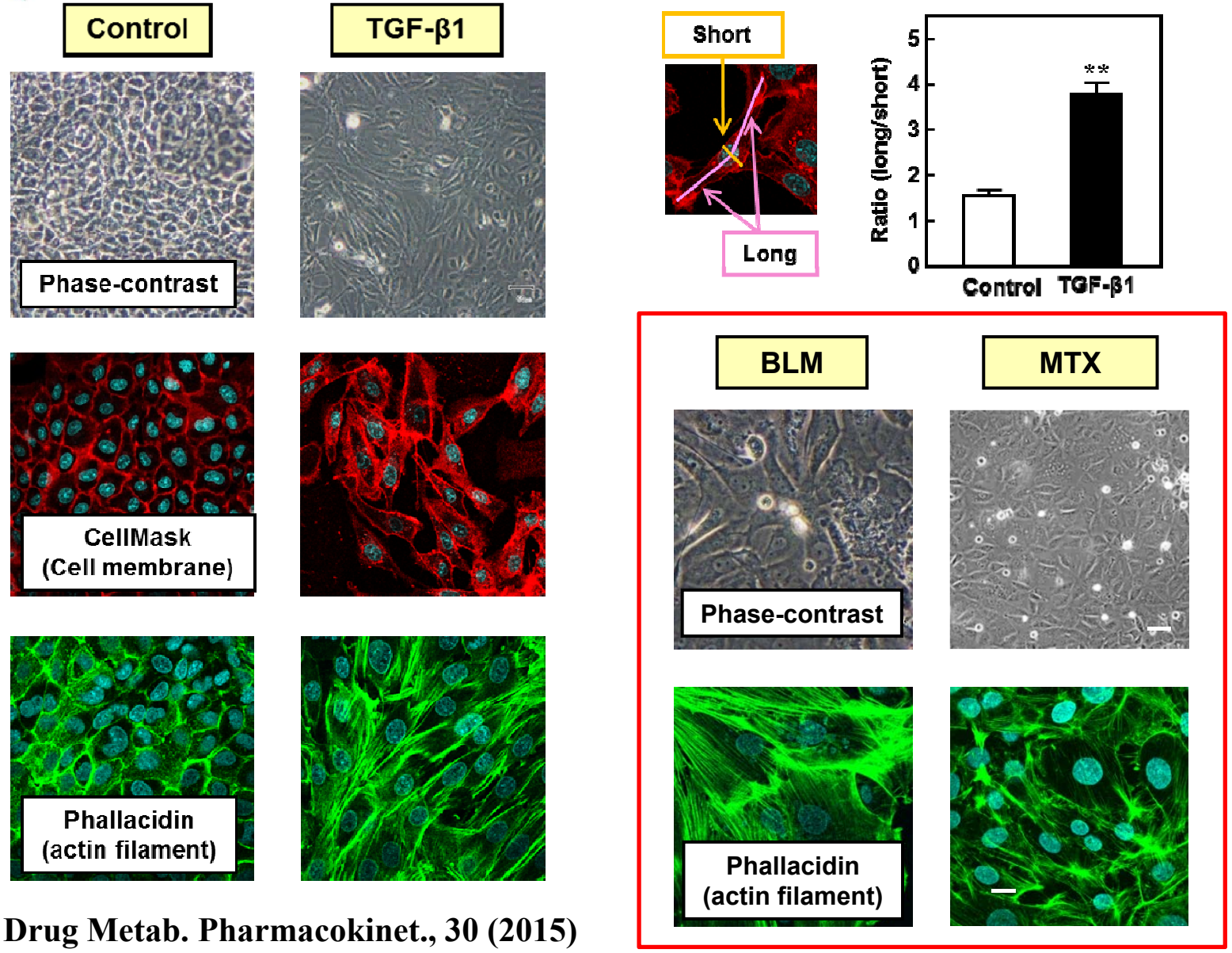
Epithelial-mesenchymal transition (EMT) of type II cells as a cause of drug-induced lung toxicity (interstitial pneumonia, pulmonary fibrosis)



Drugs w/ lung toxicity:
Anticancer drugs such as bleomycin (BLM) and methotrexate (MTX) etc.

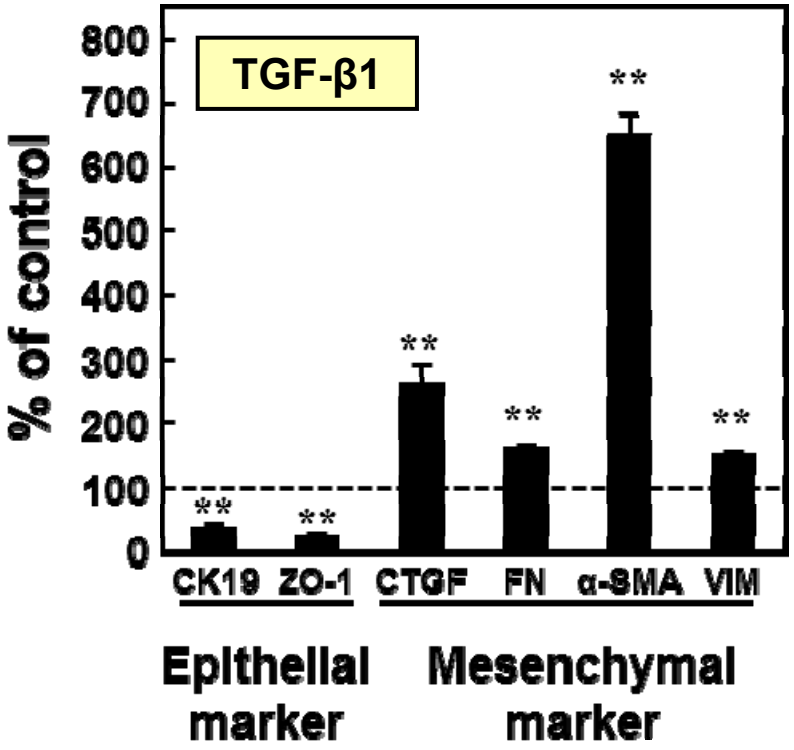


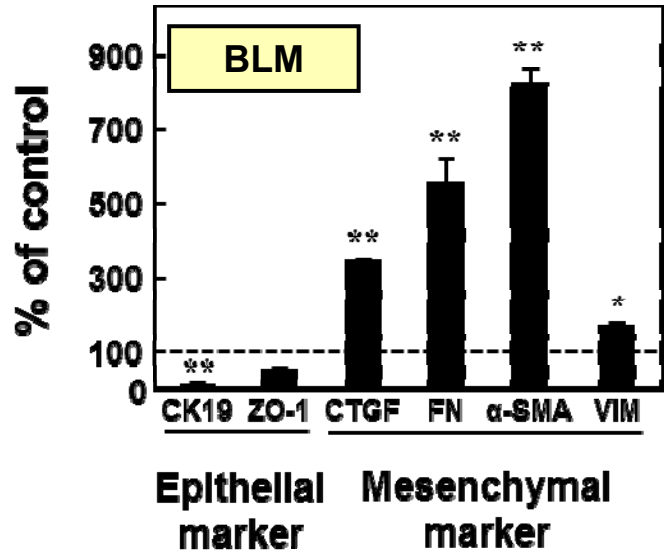
Induction of EMT by TGF-β1, bleomycin (BLM) and methotrexate (MTX) in RLE/Abca3 cells (morphology)

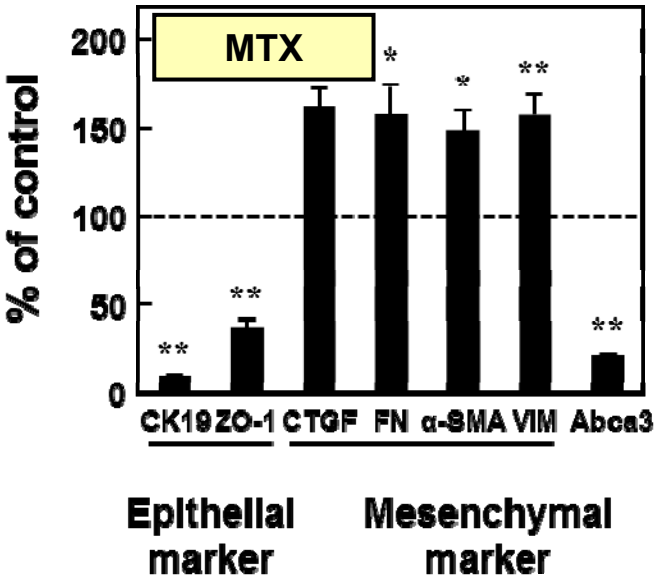


Drug Metab. Pharmacokinet., 30 (2015)

Induction of EMT by TGF-β1, bleomycin (BLM) and methotrexate (MTX) in RLE/Abca3 cells (mRNA expression)





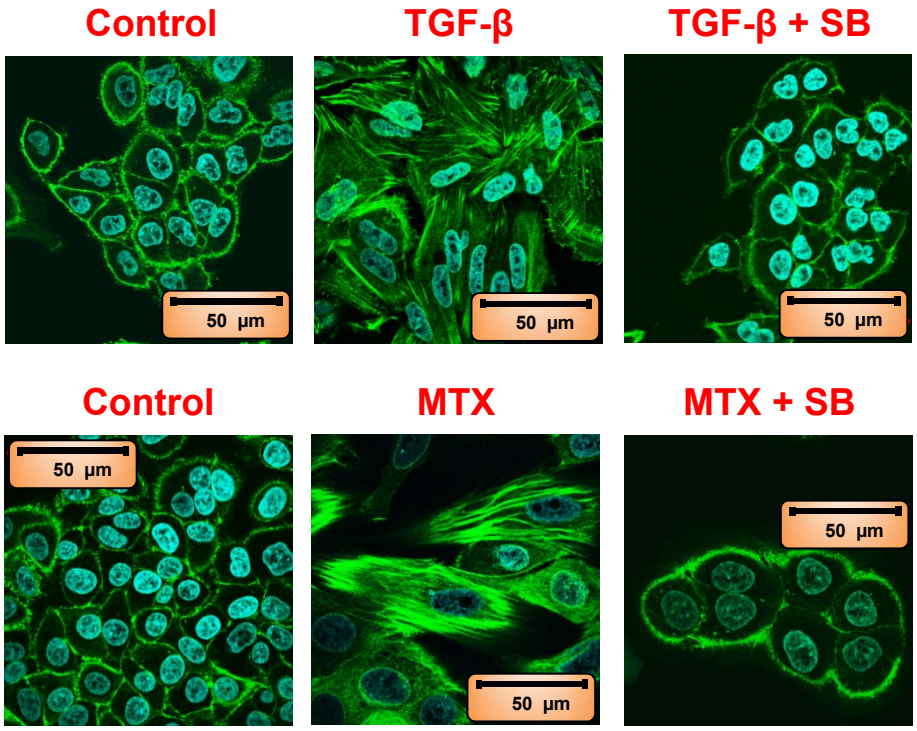


These findings suggest that, like TGF-β1, BLM and MTX induce EMT in RLE/Abca3 cells. Similar effect of MTX was observed in human-derived A549 alveolar epithelial cell line.

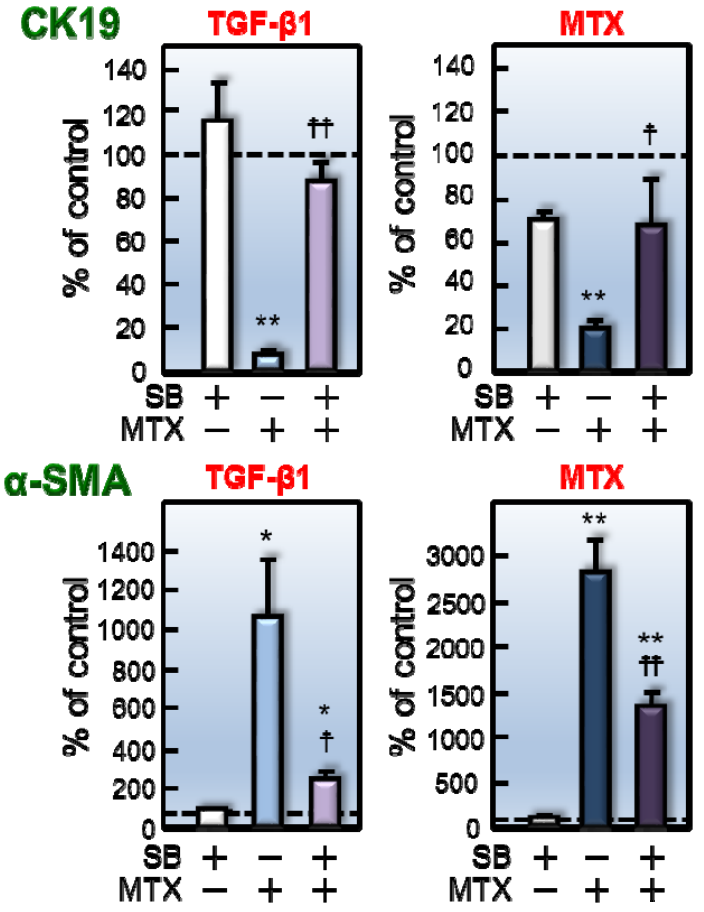
Molecular mechanism of EMT induction by MTX in A549 cells: Effect of SB on TGF-β1 and MTX-induced EMT

SB (SB431542) is a TGF-β receptor kinase inhibitor, which inhibits TGF-β signaling pathway.

Morphological changes



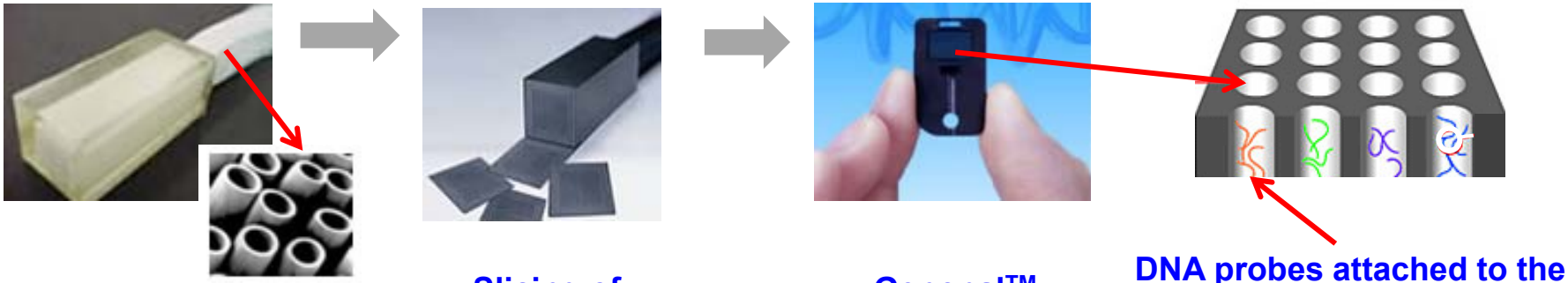
Changes in EMT-marker mRNA expression



Prediction of possible lung toxicity of drugs

Development of a novel focused microarray analysis system

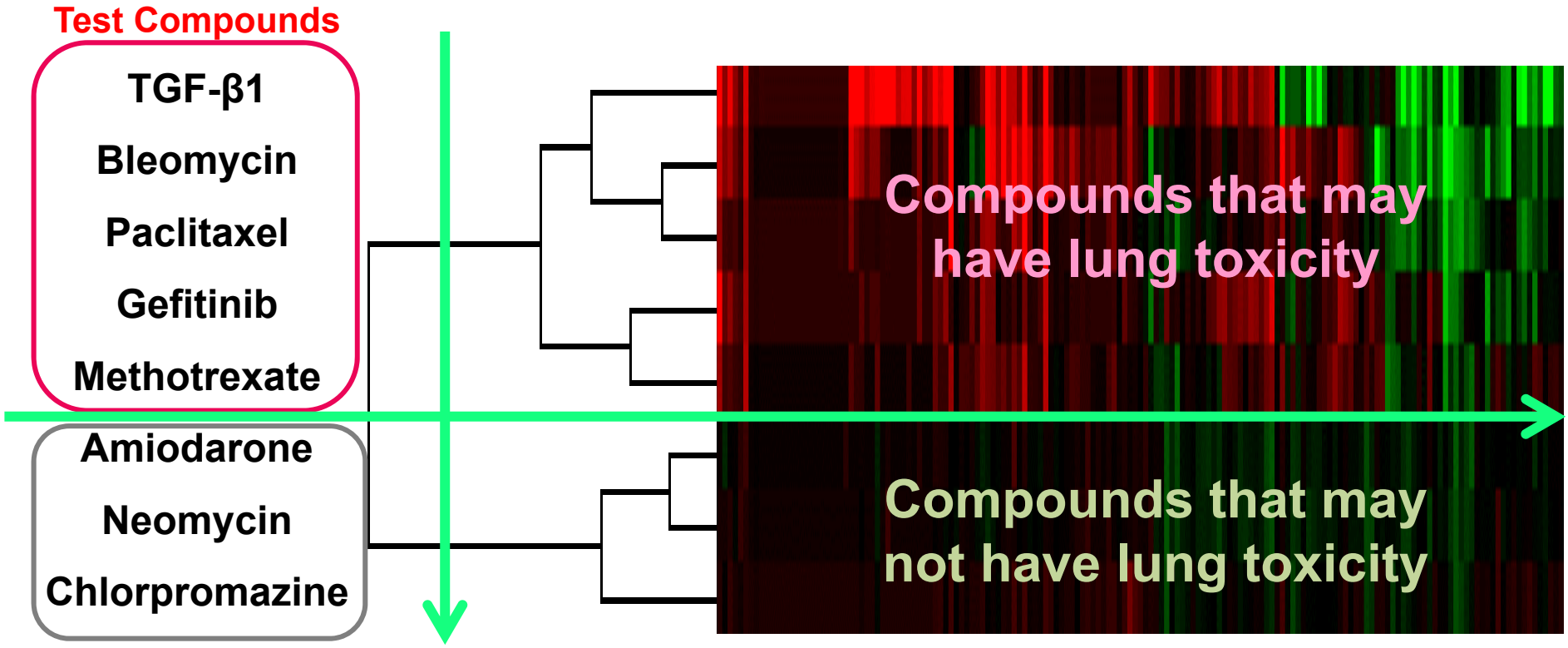
(Collaboration with Mitsubishi Rayon Co. Ltd)



In contrast to general screening microarray chips, focused microarray chips contain only about 100-200 DNA probes to detect the mRNA expression changes in a specific event, lung toxicity in this case. For this purpose, we selected 162 DNA probes based on the information concerning gene expression changes in familial and sporadic interstitial pneumonia (Yang et al., Am J Respir Crit Care Med 2007) and on our EMT studies, and prepared a novel focused microarray chip to detect possible lung toxicity of drugs/drug candidates.

Prediction of drugs having possible lung toxicity

(Focused microarray chip/Hierarchical cluster analysis)



TGF-β1, Bleomycin, Paclitaxel, Gefitinib, Methotrexate:
Lung toxicity (fibrosis)

Amiodarone:
Lung toxicity (predominantly by the metabolite of amiodarone, DEA)

Neomycin: Renal toxicity

Chlorpromazine: Liver toxicity

Summary (3)

- Bleomycin (BLM) and methotrexate (MTX) induced EMT in type II alveolar epithelial cells, which may be one of the mechanisms underlying the drug-induced pulmonary fibrosis.

- We have developed a novel focused microarray chip, and the hierarchical cluster analysis with this chip may be a useful strategy to predict possible lung toxicity (especially pulmonary fibrosis) of new drug candidates at the early stage of drug development.

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