Effects of urban $PM_{2.5}$ on airway epithelial cells *in vitro*

Expression of EGFR ligands

Mélina RUMELHARD

PhD Director: Dr. A. BAEZA
Laboratory of Cytophysiology and Cellular Toxicology
Director: Pr F. MARANO
UNIVERSITE PARIS 7

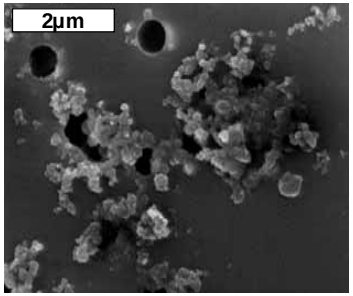
ministère éducation nationale enseignement supérieur recherche
ministère délégué à l'enseignement supérieur et à la recherche

université PARIS DIDEROT PARIS 7

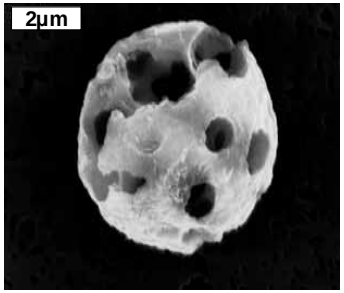
Introduction: $PM_{2.5}$

Aerodynamic diameter	Particulate Matter
<10µm	Coarse particles PM_{10}
<2.5µm	Fine particles $PM_{2.5}$
<0.1µm	Ultrafine particles $PM_{0.1}$

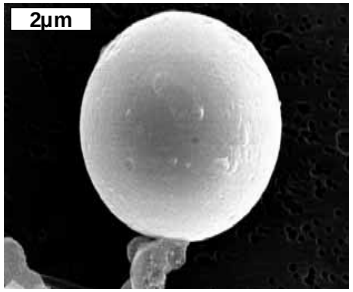
Paris $PM_{2.5}$
(from Baulig and coll., 2004, Environ. Sci. Technol.)



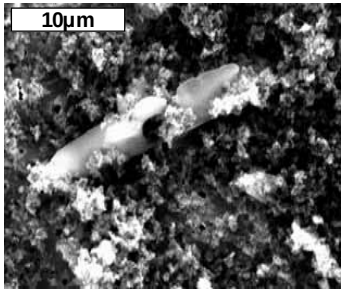
Soots



Fly ash



Fly ash



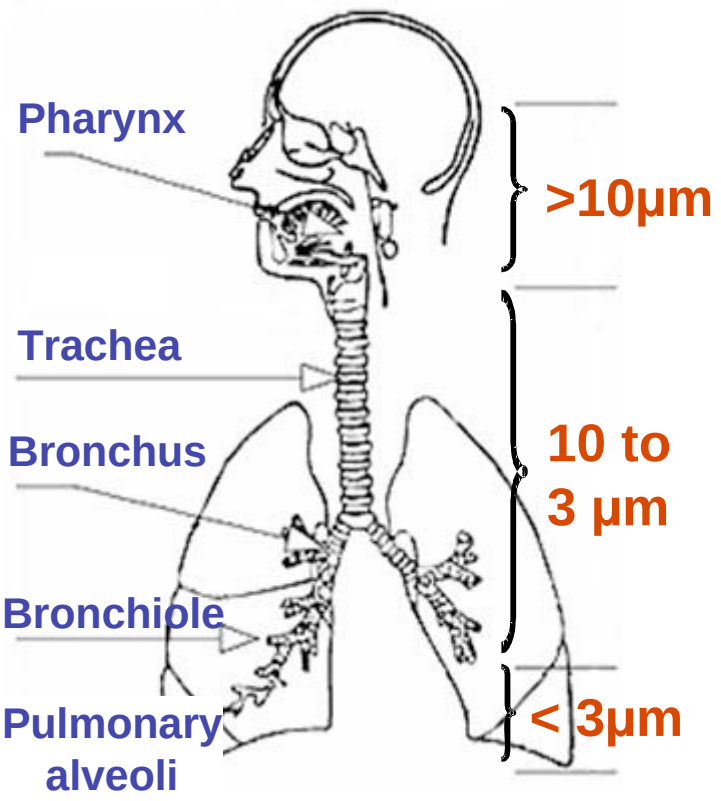
Pneumatic wear

-> Composition of $PM_{2.5}$:

- carbonaceous core
- organic compounds
- metals
- biologic compounds (endotoxins, allergens…)

Introduction: $PM_{2.5}$

Aerodynamic diameter	Particles
<10μm	Coarse particles PM_{10}
<2.5μm	Fine particles $PM_{2.5}$
<0.1μm	Ultrafine particles $PM_{0.1}$



Introduction

-> **Epidemiology:** Cardiovascular and respiratory diseases
(Pope, 2000)

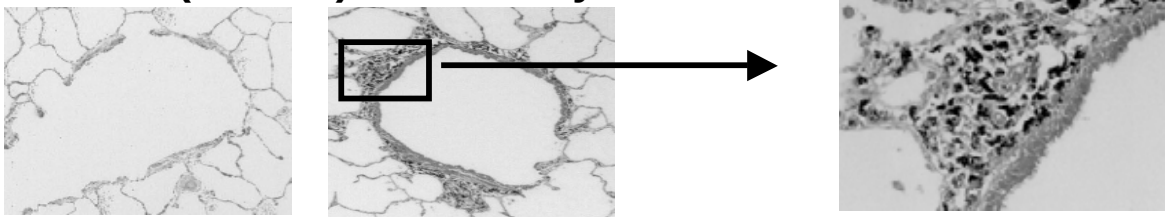
-> **Biological effects (*in vivo* studies):**

✓ airway inflammation

✓ airway remodeling

Accumulation of particles in lungs *(Brauer, 2001)*,
airway wall thickening *(Churg, 2003)*

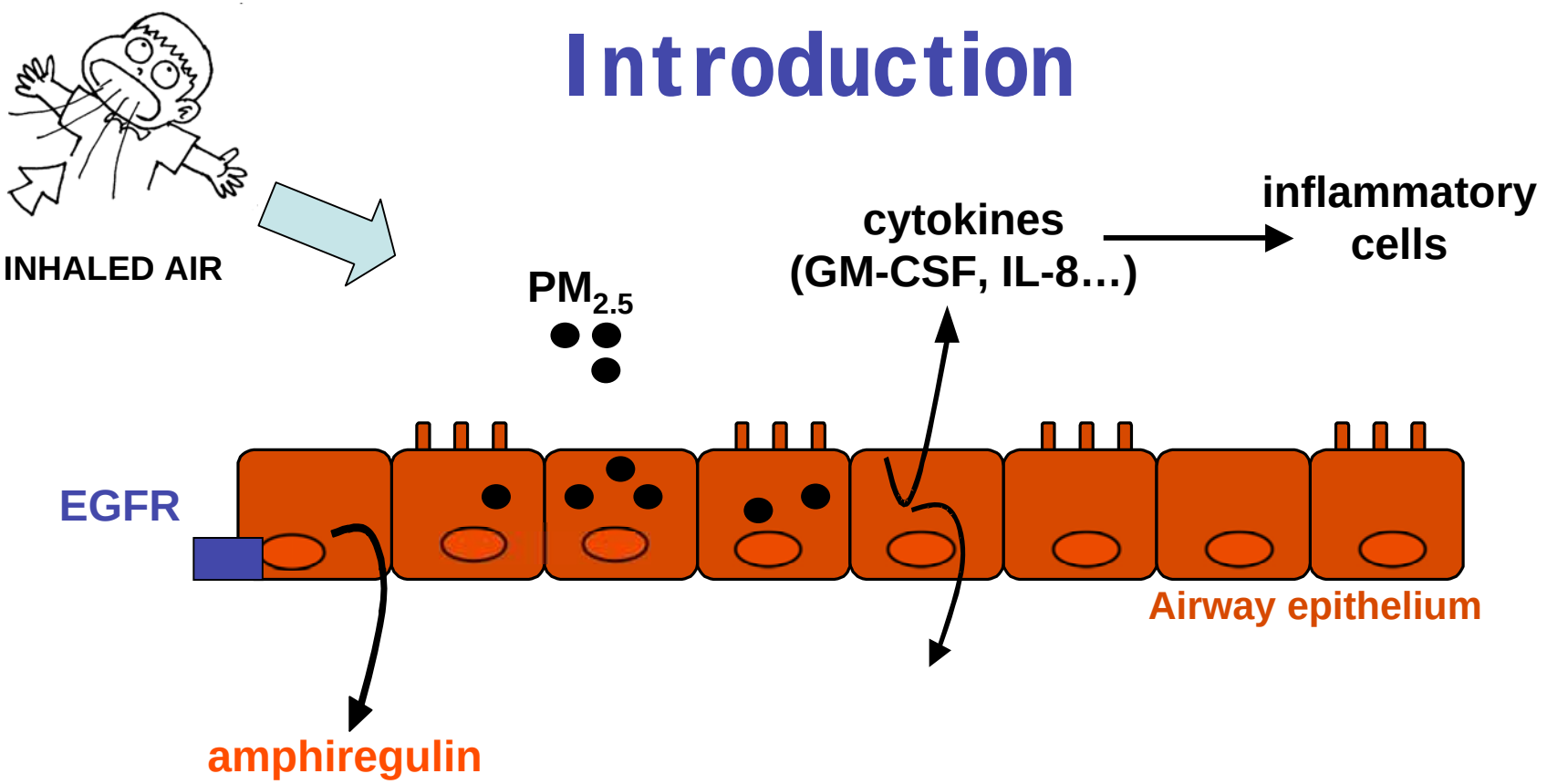
Vancouver (control) Mexico city



Pulmonary bronchioles (x 50)

x200

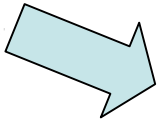
Introduction



EGF receptor ligands :
Amphiregulin, HB-EGF,
TGFα, Betacellulin

Aims of the study

INHALED AIR



EGF receptor ligands :
Amphiregulin, HB-EGF,
TGFα, Betacellulin

expression

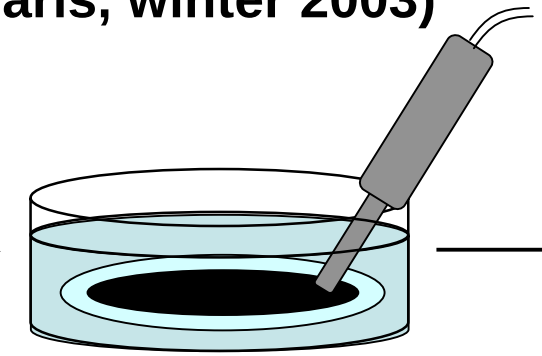
Autocrine effects of EGFR ligands?

- **on proinflammation**
- **on cell proliferation**

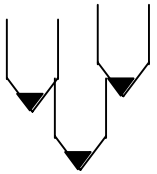
Materials and methods

-> $PM_{2.5}$ (suburb of Paris, winter 2003)

sampling

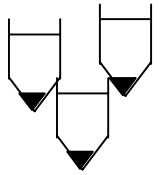


sonication

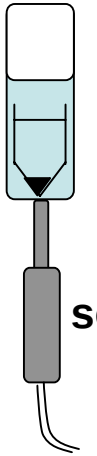


lyophilisation

2mg/mL

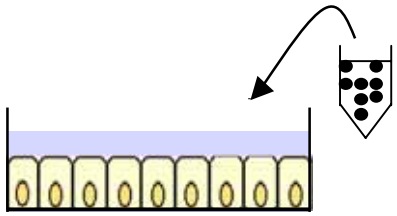


storage
-20°C



sonication

$PM_{2.5}$



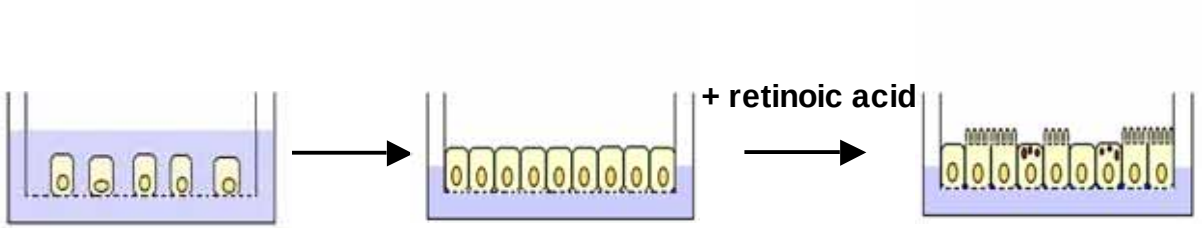
cell exposure

Materials and methods

-> Cells: ☛ 16HBE 14o- cell line ⟶ submerged in medium

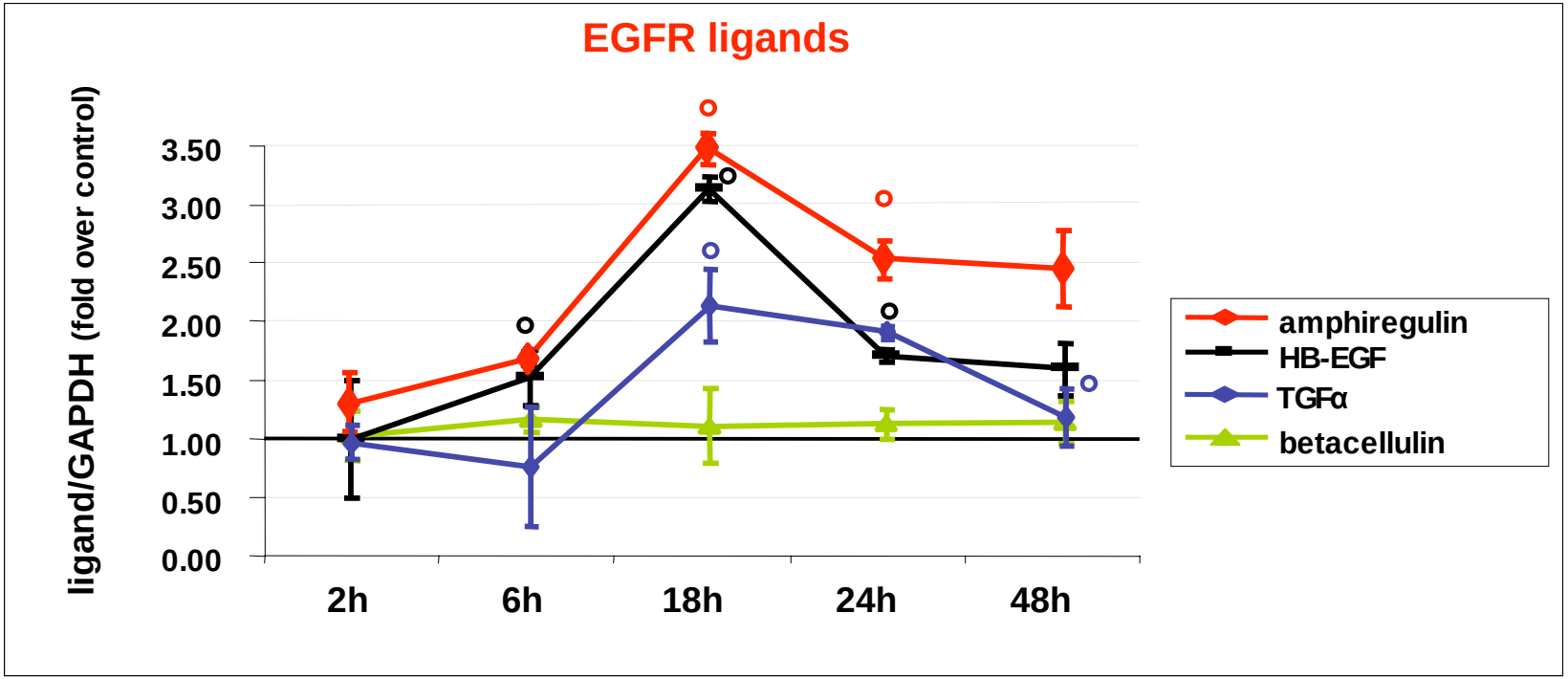
☛ normal human nasal epithelial cells

⟶ air-liquid interface (Transwell filters)



EGF receptor ligands expression

16HBE cells, real-time PCR,
$PM_{2.5}$ 10µg/cm²

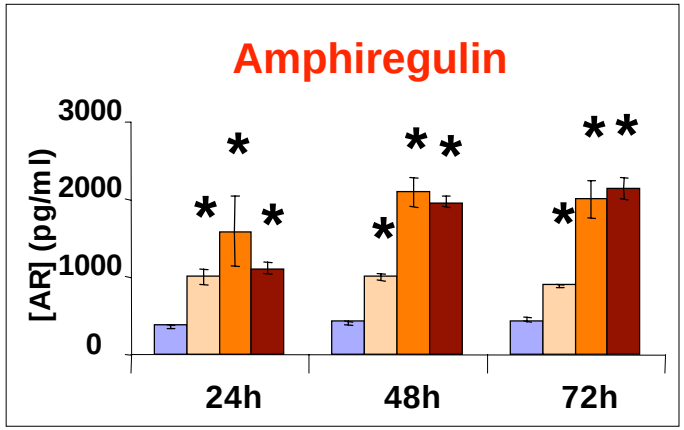


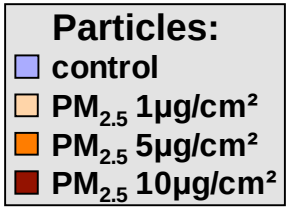
=> $PM_{2.5}$ ovexpresses EGFR ligands genes :
Amphiregulin, HB-EGF, TGFα

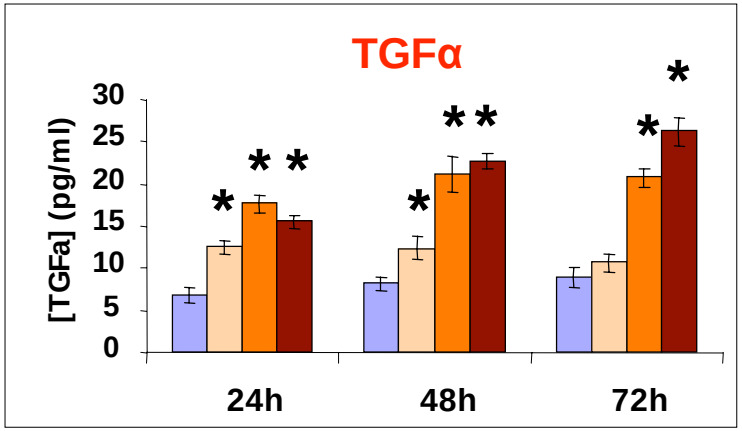
No modulation of betacellulin mRNA expression.

EGF receptor ligands release

16HBE cells (ELISA)







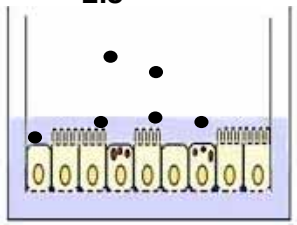
=> 2 EGFR ligands release are induced by 16HBE cells exposed to $PM_{2.5}$: amphiregulin and TGFα.

Betacellulin:
no release

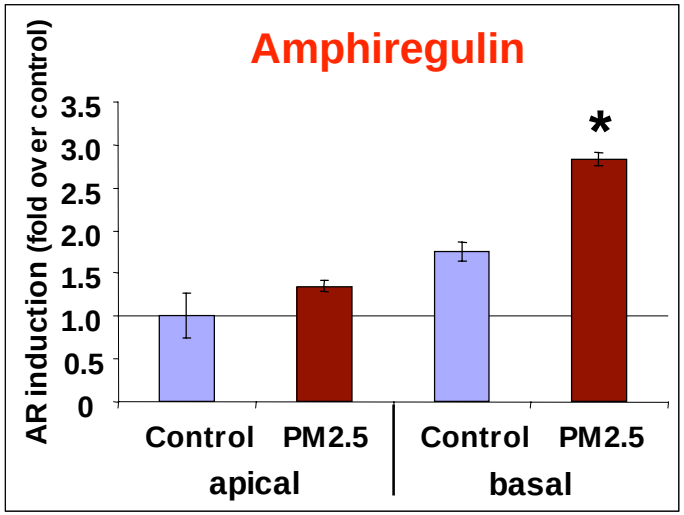
EGF receptor ligands release

Normal human nasal epithelial cells (ELISA)

$PM_{2.5}$ 10µg/cm²

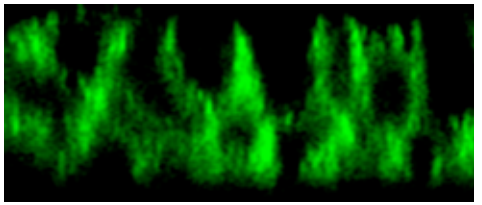


24h of exposure



Immunofluorescence
anti-EGFR Ab

apical

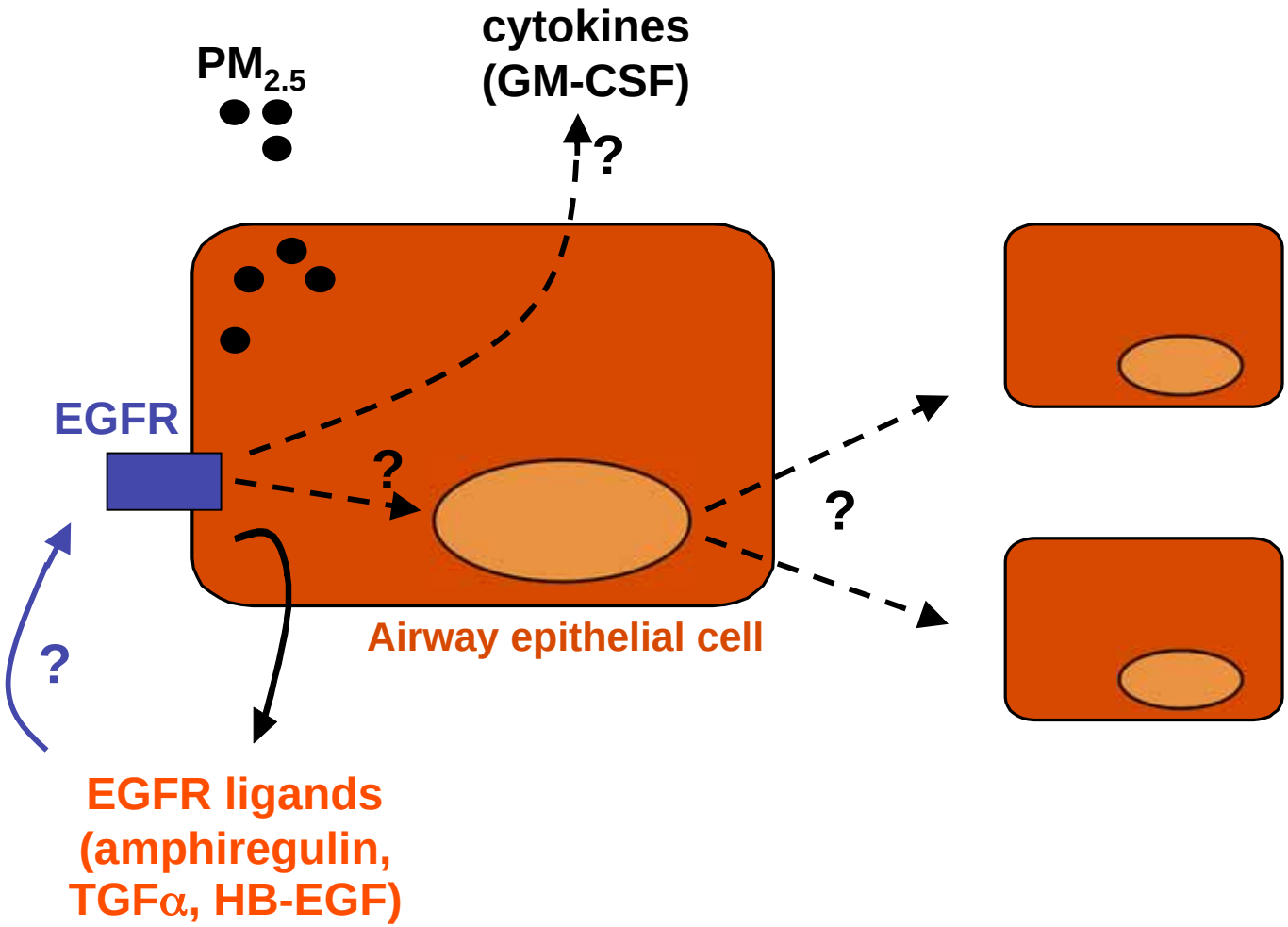


basal

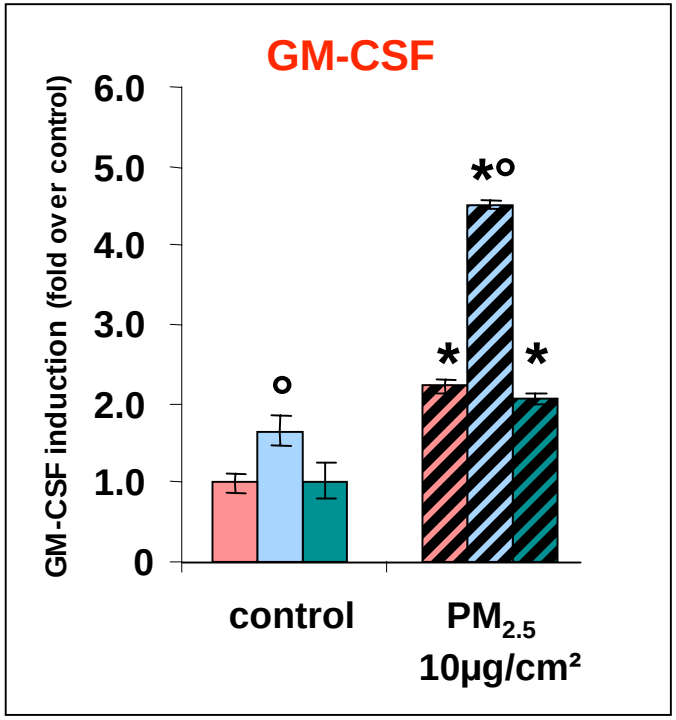
From Rumelhard and coll., Tox. Letters, revision
& Auger and coll., Toxicol. Appl. Pharmacol., 2006

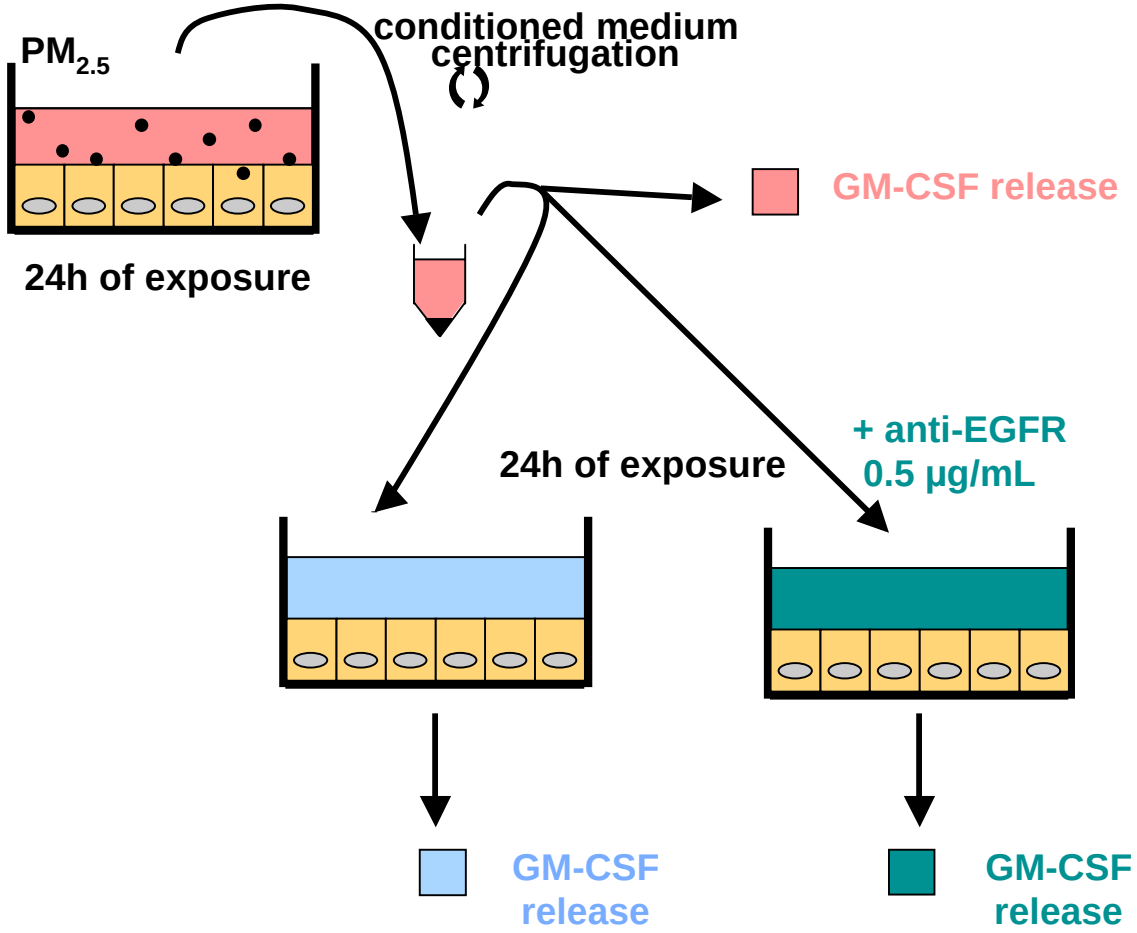
=> Amphiregulin release is induced by normal human nasal epithelial cells exposed to $PM_{2.5}$, this release is polarized towards the basolateral side where EGFR is expressed.

Conclusion (1)



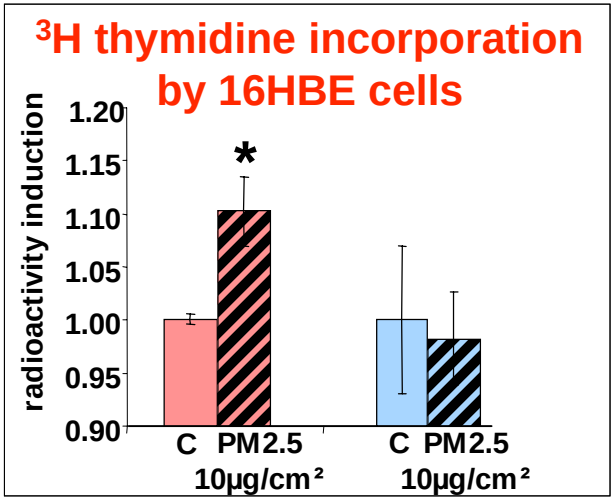
Role of EGFR ligands in bronchial proinflammatory response

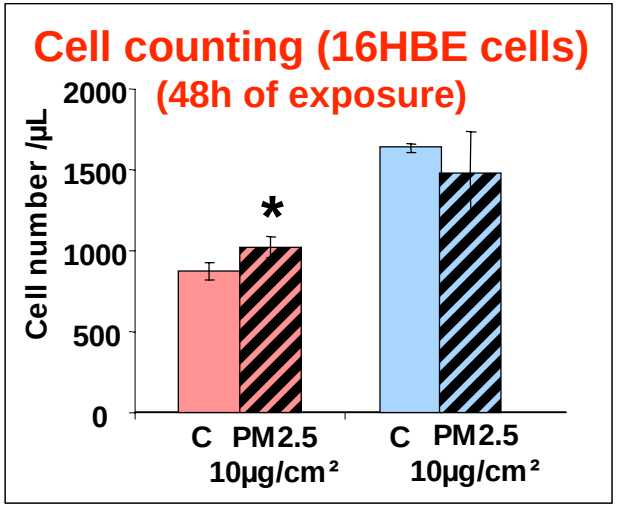


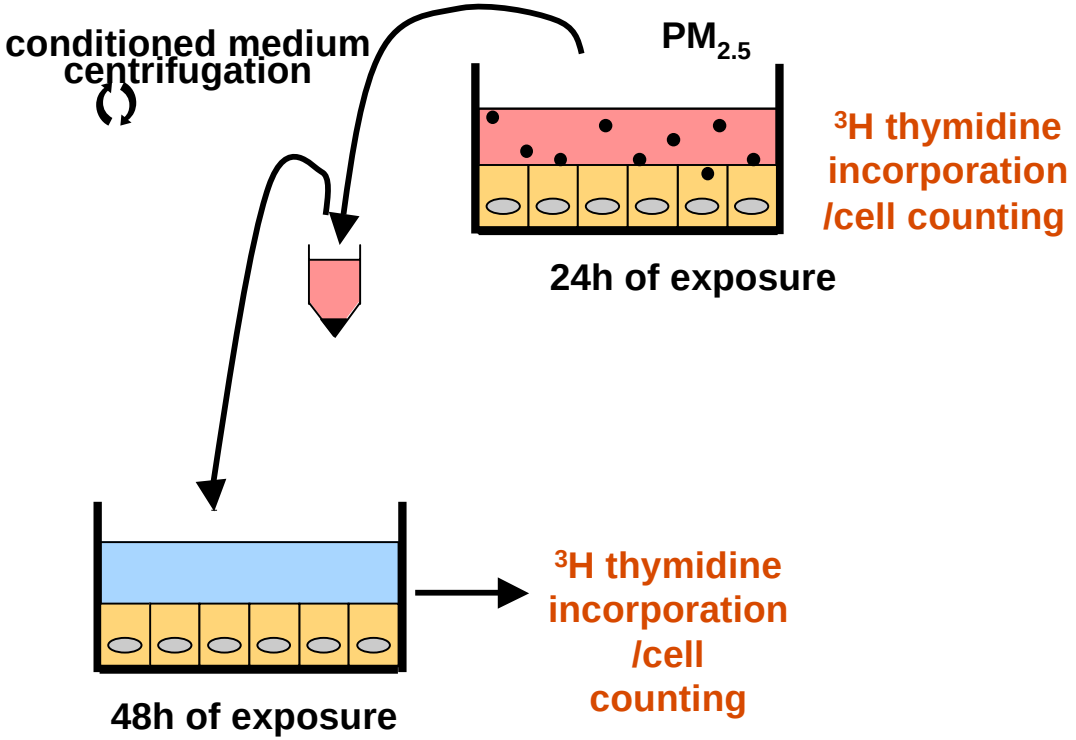


=> Autocrine induction of a proinflammatory cytokine (GM-CSF), mediated by EGF receptor ligands

Autocrine effects of $PM_{2.5}$ on bronchial epithelial cell proliferation?

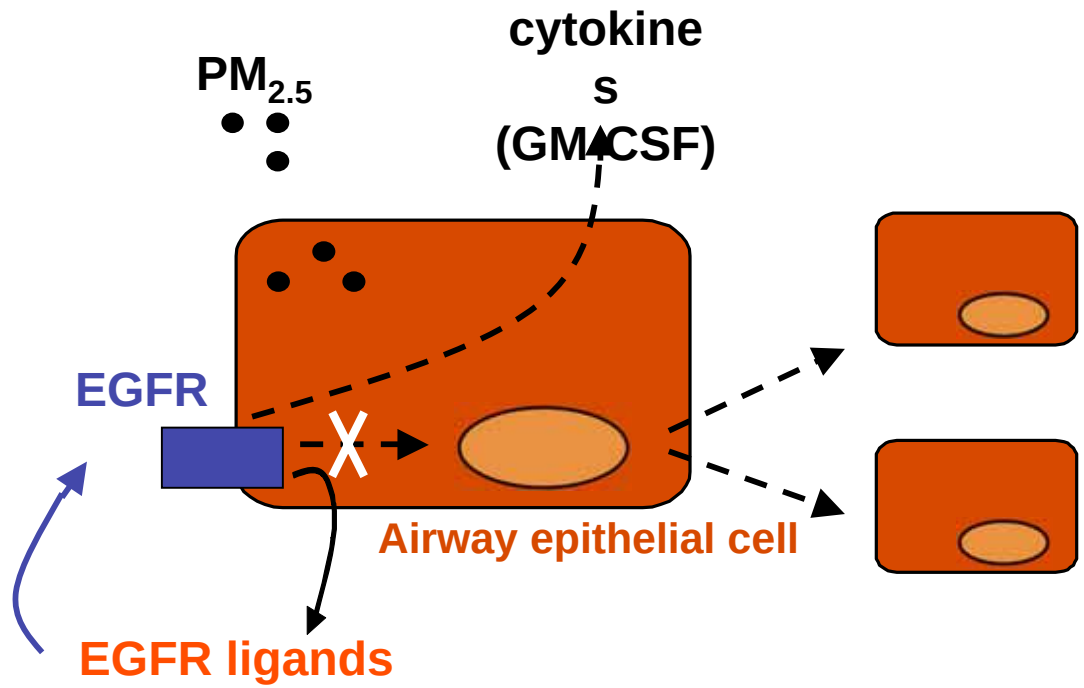




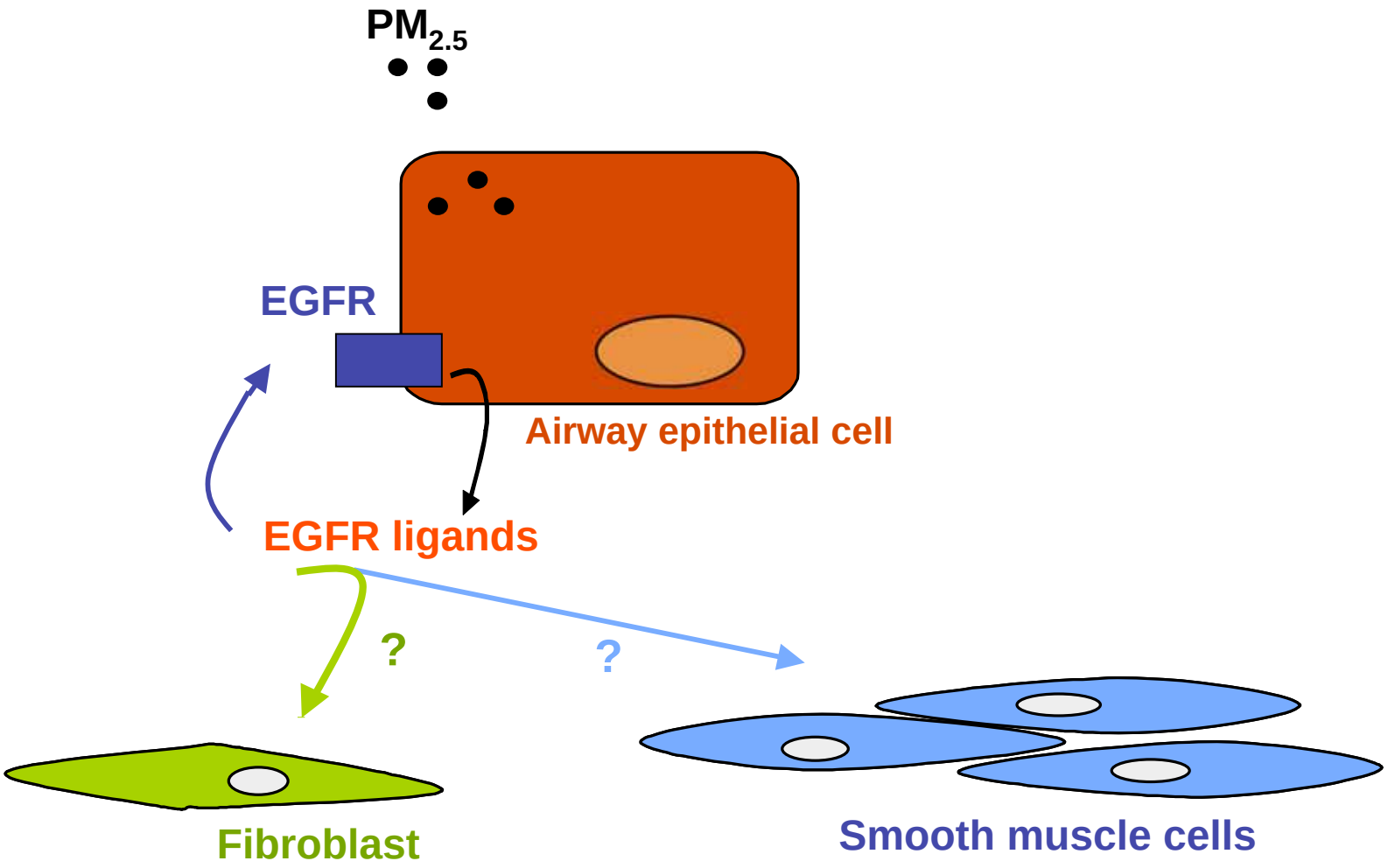


=> $PM_{2.5}$-induced secretions may not be able to provoke an autocrine bronchial epithelial cell proliferation

Conclusion (2)



Conclusion (2) - Perspectives



UNIVERSITE PARIS 7 - DENIS DIDEROT

Laboratoire de Cytophysiologie et Toxicologie cellulaire

Floriane AUGER

Anne-Catherine DAZY

Kiran RAMGOLAM

Annie JAEGER

Armelle BAEZA-SQUIBAN

Francelyne MARANO

Karine ANDREAU
Emmanuelle BOISVIEUX
Sonja BOLAND
Julien DAIROU
Jean-Marie DUPRET
Rodolphe HAMEL
Claudette JEULIN
Olja KACANSKI
Pascale LAINE
Martha MARTINS
Sandrine MIDDENDORP
Emile PETIT
Benjamin PLUVINAGE
Cécile PONSARD
Nilusha RAGUNATHAN
Fernando RODRIGUEZ-LIMA
Virginie TICHANNE-SELTZER