Nicotine Promotes Tumor Growth and Metastasis in Mouse Models of Lung Cancer

Rebecca Davis, Wasia Rizwani, Sarmistha Banerjee, Michelle Kovacs, Eric Haura, Domenico Coppola, Srikumar Chellappan
Background

Smoke ingredients
Acetone – found in nail polish remover
Acetic Acid – an ingredient in hair dye
Ammonia – a common household cleaner
Arsenic – used in rat poison
Benzene – found in rubber cement
Butane – used in lighter fluid
Cadmium – active component in battery acid
Carbon Monoxide – released in car exhaust fumes
Formaldehyde – embalming fluid
Hexamine – found in barbecue lighter fluid
Lead – used in batteries
Napthalene – an ingredient in moth balls
Methanol – a main component in rocket fuel
Nicotine – used as insecticide
Tar – material for paving roads
Toluene - used to manufacture paint
Background

Excitatorische Synapse

Epithelial markers
- E-cadherin
- Claudin
- Occludin
- Desmoglein
- Desmocollin
- Cytokeratins

EMT effectors
- Growth factors
- Cytokines
- ECM

Mesenchymal markers
- N-cadherin
- Vimentin
- Fibronectin
- Snail/2
- FSP1
- Smooth muscle actin

Characteristics
- Cobblestone
- Non-motile
- Non-invasive

Characteristics
- Elongated
- Motile
- Invasive

ACh = Acetylcholin
Background
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Methods

in vitro assays

- Bromodeoxyuridine assay
- RT-PCR
  - alpha7-nAChR
- ELISA
  - Cotinine levels
  - Noradrenaline
  - Adrenaline
- Immunohistochemistry
  - alpha7-nAChR
  - E-Cadherin
  - beta-Catenin
  - ZO-1 (zonula occludens)

- cell culture
- cell lysate
- urine
- cell supernatants (not shown)
- cell supernatants (not shown)
- excised tumors
Results

Figure 1A. Nicotine promotes the growth of Line1 cells.

Methods

BALB/c tumor growth & metastasis \textit{in vivo}

- **day 0**: 1 x 10^4 Line1 cells
- **day 7**: 1 mg/kg nicotine i.p. (3x/week)
- **day 7**: 25 mg/kg nicotine t.d. (daily)
- **day 21**: Vehicle control

Results

Figure 1BC. Nicotine promotes the growth *in vivo*.
Methods

BALB/c tumor growth & metastasis in vivo

Day 0

1 x 10^6 Line1 cells

Day 0

1 x 10^6 Line1 cells

Day 7

1 mg/kg nicotine i.p. (3x/week)

Day 21

25 mg/kg nicotine t.d. (daily)

Vehicle control

Day 21

Day 35

1 mg/kg nicotine i.p. (3x/week)

Vehicle control

Vehicle control

tumor resection

Results

Figure 2. Nicotine increases metastatic potential.

Methods

A/J tumorigenicity assay

week 0  week 5  week 33

100 mg/kg NNK (1x/week)

Goodsell D S The Oncologist 2004;9:353-354

Results

Figure 3. Nicotine increases number and size of NNK induced lung tumors.

Results

Figure 4. Nicotine enhances α7 nAChR subunit expression in Line1 cells.
Results

Figure 5. Nicotine reduced the expression of epithelial markers in A/J mice.

Conclusion

- Fatality of on-going smoking in cancer patients
- Promotion of tumor re-growth and metastasis via i.p. & trans-dermal nicotine
Limitations

• Cotinine levels:
  • i.p. group: 3000 ng/mL
  • patch group: 5000 ng/mL

  „doses used in these studies correlated well with cotinine levels in urine of heavy smokers“

• Local recurrence
  • complete resection achieved? Histology?

• Inconsistent findings - Comparability between assays (different cell passages)