15-lipoxygenases and their metabolites as biomarkers for the early detection of smoking-induced non-small cell lung cancer

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Incidence of lung cancer

<table>
<thead>
<tr>
<th>Site</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung &amp; bronchus</td>
<td>29%</td>
<td>26%</td>
</tr>
<tr>
<td>Prostate</td>
<td>11%</td>
<td>15%</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>9%</td>
<td>9%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>6%</td>
<td>7%</td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>4%</td>
<td>5%</td>
</tr>
<tr>
<td>Leukemia</td>
<td>4%</td>
<td>4%</td>
</tr>
<tr>
<td>Esophagus</td>
<td>4%</td>
<td>3%</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>4%</td>
<td>3%</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>3%</td>
<td>2%</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>3%</td>
<td>2%</td>
</tr>
<tr>
<td>All other sites</td>
<td>23%</td>
<td>24%</td>
</tr>
</tbody>
</table>

Source: American Cancer Society, 2010.
Etiology of lung cancer

Smokers
1. Smoking carcinogens: nicotine and its derivative N-nitrosamines, such as nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosonornicotine (NNN). - associated with 70-90% of lung cancer.
2. Genetic factor.
3. Other unidentified factors.

Non-smokers
1. Environmental: environmental tobacco smoke (ETS) or environmental smoke (ES) such as passive/second-hand smoking and emissions from high-temperature frying. - the major factor.
3. Genetic: family history, racial differences.
4. Viral: human papilloma virus (HPV), jazz siekte sheep retrovirus (JSRV).
5. Dietary.
7. Previous lung diseases.
Histologic classification of lung cancer

Non-small cell lung carcinoma (NSCLC) (~80%)
- Adenocarcinoma (40%)
- Large cell carcinoma (25%)
- Squamous cell carcinoma (10%)
- Others (adenosquamous carcinoma, sarcomatoid carcinoma) (<5%)

Small cell lung carcinoma (SCLC) (~20%)

Other types of lung cancers (<1%)
- Lung carcinoid cancer
- Adenoid cystic carcinomas
- Hamartomas
- Lymphomas
- Sarcomas
Lung cancer histologic types related to smokers and non-smokers
15-lipoxygenases (LOXs) are members of non-heme iron-containing dioxygenases. In human, 2 isoforms:
15-LOX1 (15-LOa)
15-LOX2 (15-LOb)
In mice, only one form: 12/15-LOX - the murine ortholog to human 15-LOXs.
Linoleic acid (LA) → 15-LOX-1 → 13(S)–HODE (hydroxyotadecadienoic acid)

Arachidonic acid (AA) → 15-LOX-1 → 15(S)-HETE (hydroxyeicosatetraenoic acid)
Why were 15-lipoxygenases and their metabolites selected for the study?

The metabolites of 15-lipoxygenases, 15S-HETE and 13S-HODE, are the endogenous ligands of peroxisome proliferator-activated receptor gamma (PPARγ), whose activity is significantly reduced in lung cancer, particularly, smoking-related NSCLC.

Levels of 15(S)-HETE, 13(S)-HODE, 15-LOX-1 and 15-LOX-2 in human lung tissues

(a)

Levels of 15(S)-HETE and 13(S)-HODE in non-tumor and tumor tissues.

(b)

Percentage of 15-LOX-1 and 15-LOX-2 in non-tumor and tumor tissues.
NNK-induced lung tumors in A/J mice

6 weeks old
A single dose of NNK 100mg/kg Peritoneal injection (i.p.)

Week 20-24
Epithelial/alveolar hyperplasia

Week 34
Adenocarcinoma

Week 38
End of experiments

PBS control

NNK treatment

NNK: 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone, a major cigarette smoking carcinogen.
Histopathology of lungs in control and NNK-treated group
Levels of 15(S)-HETE and 13(S)-HODE in lung tissues of A/J mice
Levels of 12/15-LOX in lung tissues of A/J mice
(12/15-LOX is ortholog to human 15-LOXs)
Expression of PPARγ protein and PPARγ transcriptional activity during NNK-mediated lung tumorigenesis in A/J mice
Levels of 15(S)-HETE and 13(S)-HODE in lung tissues of A/J mice
Serum 15S-HETE and 13S-HODE in patients with NSCLC
Correlation between Serum 15S-HETE/13S-HODE and tissue 15S-HETE/13S-HODE in patients with NSCLC
Effects of 15(S)-HETE and 13(S)-HODE on the proliferation of human NSCLC cells

- **a**: Cell proliferation of NCI-H23 cells treated with varying concentrations of 15(S)-HETE at 24, 48, and 72 hours.
- **b**: Cell proliferation of NCI-H460 cells treated with the same conditions.
- **c**: Cell proliferation of NCI-H23 cells treated with 13(S)-HODE at 24, 48, and 72 hours.
- **d**: Cell proliferation of NCI-H460 cells treated with 13(S)-HODE at the same time points.
Induction of apoptosis by 15(S)-HETE and 13(S)-HODE – sub-G1 population

**p=0.0012**

**p=0.009**

*p=0.032*

**p=0.005**

*p=0.016*

*p=0.047*

**p=0.012**

*p=0.042*
Induction of apoptosis by 15(S)-HETE and 13(S)-HODE – caspases in NCI-23
Induction of apoptosis by 15(S)-HETE and 13(S)-HODE – caspases in NCI-460
Restoration of 15-LOX-1 and 15-LOX-2 increases the levels of 15(S)-HETE and 13(S)-HODE.
Conclusions

1. The levels of 15-LOX-1 and -2 were significantly decreased in lung tissues of human NSCLC compared with the matched non-tumor lung tissues.

2. The levels of 15S-HETE and 13S-HODE, the metabolites of 15-LOX-1 and -2, were reduced in the blood of NSCLC patients compared with normal subjects.

3. The reduction of 15-LOX, 15S-HETE and 13S-HODE predated the appearance of mouse lung tumor induced by tobacco smoking.

4. 15(S)-HETE and 13(S)-HODE or 15-LOX-1 and 15-LOX-2 can inhibit the proliferation and growth of human NSCLC cells.

5. Strategies to restore 15-LOXs activities and increase the production of endogenous 15(S)-HETE and 13(S)-HODE may offer a novel research direction for the molecular targeting treatment and prevention for smoking-related NSCLC.
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