Analysis of *Kras*, *Egfr* and *Tp53* Mutations in F344/NTac Rat and B6C3F1/N Mouse Alveolar/bronchiolar Carcinomas Resulting from Chronic Inhalation Exposure to Cobalt metal

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NTP Technical Reports Peer Review Meeting
October 29, 2013
Introduction

• Mutational patterns of carcinogens
  – Tobacco smoke - Lung cancer (C:G > A:T)
  – Aflatoxin - Hepatocellular carcinoma (C:G > A:T)
  – UV light - Melanoma (C:G > T:A)
  – *H. pylori* - Gastric carcinoma (C:G > T:A)
  – *O. viverrini* - Cholangiocarcinoma (C:G > T:A)
  – Aristolochic acid - Urothelial carcinoma (A:T > T:A)

*Poon et al., 2013; Lee and Ladanyi, 2013*
Introduction

• “Driver” mutations in genes of human lung cancer
  – KRAS, EGFR, ALK, ERBB2, BRAF, MAP2K1, PIK3CA, FGFR1, MET, DDR2
  – TP53, PTEN, STK11, AKT1

• Most commonly altered and evaluated mutations in human Non Small Cell Lung Carcinoma (NSCLC) include KRAS, EGFR and TP53

Imielinski et al., 2012; Pao and Girard, 2011
Introduction

• Human Non Small Cell Lung Carcinoma (NSCLC)
  – *KRAS* mutations (26%; 67/254)
  – *EGFR* mutations (9%; 22/254)
  – *TP53* mutations (50%; 52/104)

• *Kras* mutations in Mouse (B6C3F1) lung tumors
  – Spontaneous (27%; 34/124)
  – 1,3-Butadiene (83%; 20/24)
  – Cumene (87%; 45/52)
  – Cobalt sulfate heptahydrate (35%; 9/26)
  – Ethylene oxide (100%; 23/23)

*Sills et al., 1995; Sills et al., 1999; Hong et al., 2007; Hong et al., 2008; Boch et al., 2013; Husgafvel-Pursiainen and Kannio., 1996*
Objective

Evaluate mutations in *Kras*, *Egfr*, and *Tp53* genes in F344/NTac rat and B6C3F1/N mouse Alveolar/bronchiolar carcinomas (ABCs) arising spontaneously (in controls) and by chronic inhalation exposure to Cobalt metal.
Materials and methods

• DNA was extracted from formalin fixed paraffin embedded (FFPE) ABC tissues from the 2-year bioassay
  – Tumors >5 mm were razor-dissected from five 10 micron FFPE sections
  – If the tumors were microscopic and randomly scattered, then entire FFPE sections were used for DNA extraction

• Semi-nested PCR
  – Kras (exons 1 and 2)
  – Egfr (exons 18-21)
  – Tp53 (exons 5-8)

• Amplified DNA purified and Sanger sequenced (2x)

• Samples with mutations were confirmed by repeat analysis starting with the original DNA extracts.
Results
Rat ABC mutation analysis

<table>
<thead>
<tr>
<th>Cobalt metal</th>
<th>n</th>
<th>Kras</th>
<th>Egfr</th>
<th>Tp53</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control#</td>
<td>10</td>
<td>0 (0%)**</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>1.25 mg/m³</td>
<td>14</td>
<td>2 (14%)</td>
<td>2 (14%)</td>
<td>3 (21%)</td>
</tr>
<tr>
<td>2.5 mg/m³</td>
<td>17</td>
<td>6 (35%)*</td>
<td>3 (18%)</td>
<td>6 (35%)*</td>
</tr>
<tr>
<td>5 mg/m³</td>
<td>17</td>
<td>7 (41%)*</td>
<td>3 (18%)</td>
<td>2 (12%)</td>
</tr>
<tr>
<td>Treated Total</td>
<td>48</td>
<td>15 (31%)*</td>
<td>8 (17%)</td>
<td>11 (23%)</td>
</tr>
</tbody>
</table>

* Significantly different (P≤0.05) from the chamber control group by the Fisher’s exact test
** Significant trend (P≤0.001) by the Cochran-Armitage trend test
# Spontaneous alveolar/bronchiolar carcinomas (n=10) were sourced from vehicle or chamber control groups in various NTP chronic bioassays.
Results
Mouse ABC mutation analysis

<table>
<thead>
<tr>
<th>Cobalt metal</th>
<th>n</th>
<th>Kras</th>
<th>Egfr</th>
<th>Tp53</th>
</tr>
</thead>
<tbody>
<tr>
<td>Historical control#</td>
<td>124</td>
<td>34 (27%)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>0 (0%)####</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>1.25 mg/m³</td>
<td>16</td>
<td>11 (69%)##</td>
<td>2 (13%)</td>
<td>3 (19%)</td>
</tr>
<tr>
<td>2.5 mg/m³</td>
<td>23</td>
<td>11 (48%)##</td>
<td>7 (30%)</td>
<td>3 (13%)</td>
</tr>
<tr>
<td>5 mg/m³</td>
<td>30</td>
<td>24 (80%)##</td>
<td>3 (10%)</td>
<td>7 (23%)</td>
</tr>
<tr>
<td>Treated Total</td>
<td>69</td>
<td>46 (67%)##</td>
<td>12 (17%)</td>
<td>13 (19%)</td>
</tr>
</tbody>
</table>

** Significantly different (P≤0.01) from the chamber control group by the Fischer’s exact test
*** P≤0.001 by one-sided Fischer exact test for single or combined exposure groups or a one-sided Cochran-Armitage trend test for the chamber control group
### Significant trend (P≤0.001) by the Cochran-Armitage trend test
# All routes, all vehicles; NA = not available
Results

- **Kras mutations**
  - Rats (n=15): codon 12 (93%) > codon 13 (7%)
  - Mice (n=48*): codon 12 (63%) > codon 61 (29%) > codon 13 (8%)
  - Mice historical control spontaneous ABC (n=34)
    - codon 12 (59%) > codon 61 (23%) > codon 13 (18%)

- **Egfr mutations**
  - Rats (n=9*): exon 20 (67%) > exon 21 (22%) > exon 19 (11%)
  - Mice (n=12): exon 20 (50%) > exon 21 (33%) > exon 18 & 19 (8%)

- **Tp53 mutations**
  - Rats (n=13*): exon 6 (38%) > exon 7 and 8 (23%) > exon 5 (15%)
  - Mice (n=14*): exon 5 (50%) > exon 7 (29%) > exon 6 (21%)

* Double mutations included
Results

• *Kras* mutations
  - Rats: G→T transversions (57%; 8/14) and G→A transitions (43%; 6/14)
  - Mice: G→T transversions (80%; 24/30) and G→A transitions (17%; 5/30)
  - Mice historical spontaneous ABC (n=124): G→A transition (70%; 14/20)

• *Egfr* mutations
  - Rats: Transitions G→A (50%; 5/10) or C→T (30%; 3/10)
  - Mice: Transitions G→A (42%; 5/12) or C→T (17%; 2/12)

• *Tp53* mutations
  - Rats: Transitions C→T (38%; 5/13) or G→A (31%; 4/13)
  - Mice: Transversions G→C (60%; 9/15)
Discussion

- *Kras* Codon 12 mutations were the most common mutations in rat and mouse ABCs resulting from cobalt metal exposure
  - human NSCLC contains *KRAS* mutations in codons 12 (86%) and 13 (14%)
- G→T transversions were the most common mutations in cobalt metal exposed rat (57%) and mouse (80%) ABCs
  - one of the most common mutations in mouse (55%) ABCs resulting from cobalt sulfate heptahydrate aerosol inhalation exposure (NTP TR 471)
  - one of the most common mutations in human (67%) NSCLC
  - correlate with 8-OHdG adducts resulting from oxidative stress

*Sills et al., 1998 in NTP TR 471; Devereux et al., 1993; Rodenhuis et al., 1989; Siegfried et al., 1997*
Acknowledgements

• Lily Hong
• Mark Hoenerhoff
• Ronald Herbert
• Robert Sills
• Michelle Hooth
• Mamta Behl
• Grace Kissling
• Kristine Witt
• NIEHS sequencing core