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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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

		Mutation Frequency		
Cobalt metal	**n**	***Kras***	***Egfr***	***Tp53***
Control#	10	0 (0%)**	0 (0%)	0 (0%)
1.25 mg/m^3	14	2 (14%)	2 (14%)	3 (21%)
2.5 mg/m^3	17	6 (35%)*	3 (18%)	6 (35%)*
5 mg/m^3	17	7 (41%)*	3 (18%)	2 (12%)
Treated Total	**48**	**15 (31%)***	**8 (17%)**	**11 (23%)**

***** 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

		Mutation Frequency		
Cobalt metal	**n**	***Kras***	***Egfr***	***Tp53***
Historical control[#]	124	34 (27%)	NA	NA
Control	10	0 (0%)[###]	0 (0%)	0 (0%)
1.25 mg/m^3	16	11 (69%)***	2 (13%)	3 (19%)
2.5 mg/m^3	23	11 (48%)**	7 (30%)	3 (13%)
5 mg/m^3	30	24 (80%)***	3 (10%)	7 (23%)
Treated Total	**69**	**46 (67%)*** **	**12 (17%)**	**13 (19%)**

** 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

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