Overview of Genomic Studies on Rodent Tumors and Its Translational Relevance

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Division of the National Toxicology Program
National Institute of Environmental Health Sciences

NTP Board of Scientific Counselors Meeting
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Overview

• A recap on molecular studies in rodent tumors in the NTP

• Goals of genomic studies on rodent tumors from NTP studies

• Progress on genomic studies on mouse hepatocellular carcinomas from NTP studies

• NTP collaborations

• Potential future studies and collaborations
• A recap on molecular studies in rodent tumors in the NTP

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• Potential future studies and collaborations
Comparisons of Leading Sites of New Cancer Cases in Humans to Tumor Sites in Animal Models

- **Male**
  - Lung
  - Prostate – Uncommon
  - Intestine
  - Pancreas
  - Liver
  - Lymphoma
  - Non-Hodgkin’s Lymphoma
  - Esophagus – Uncommon
  - Urinary Bladder
  - Kidney

- **Female**
  - Lung & Bronchus
  - Breast
  - Colon & Rectum
  - Intestine
  - Ovary
  - Non-Hodgkin’s Lymphoma
  - Leukemia
  - Uterine Corpus
  - Brain
  - Liver

Molecular Studies on NTP Rodent Tumors - A Recap

• Goals
  – Translational relevance of rodent tumors for human health
  – Mechanisms of tumors arising spontaneously or due to chemical exposures
  – Inclusion of the molecular data in NTP technical reports

• Examples
  – Chloroprene and isoprene caused similar \textit{Kras} mutations in mouse lung tumors that are distinct from mutations in 1,3-butadiene exposures (Sills \textit{et al.}, 1999)

  – \textit{Kras}, \textit{Egfr} and \textit{Tp53} mutations in mouse and rat lung tumors from cobalt metal and cobalt sulfate heptahydrate exposures (Hong \textit{et al.}, 2015)

  – Alterations in MAPK, WNT, and TGF-\(\beta\) signaling in large intestinal tumors in rats exposed to Aloe vera extract (Pandiri \textit{et al.}, 2011)
Histopathology of the large intestinal tumors in rats exposed to AVNWLE

AVNWLE-induced large intestinal tumors in F344/NCTR rats have morphological features similar to human colon cancer.

Colon adenocarcinoma - Rat

Colon adenocarcinoma - Human

Hisatsune et al., 2013
Molecular Alterations in Human Colorectal Cancer

Molecular Alterations in Human Colorectal Cancer

**Mutation Analysis using Sanger Sequencing**

Comparison of mutation frequencies in AVNWLE-induced large intestinal tumors in rats with human colorectal cancer (CRC) and other rat CRC models

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<tr>
<th>Group</th>
<th>% Ctnnb1 mutations</th>
<th>% Kras mutations</th>
<th>% Tp53 mutations</th>
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<td>AVNWLE</td>
<td>33</td>
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<td>Human CRC</td>
<td>15-26</td>
<td>40-60</td>
<td>50*</td>
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<td>Azoxymethane</td>
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<td>Heterocyclic Amines</td>
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PCR arrays - WNT, MAPK, TGF-β pathways

Clustering of normal colon, adenoma and carcinoma samples based on WNT, MAPK, TGF-β pathway directed gene expression profiles

Principal Components Analysis

Hierarchical Cluster Analysis
Altered molecular pathways

- 39/84 genes: Wnt/Ctnnb1 pathway
- 24/84 genes: MAPK pathway
- 24/32 genes: TGF-β pathway
- 33/60 genes: Other genes in hCRC
Rat Large Intestinal Tumors vs. Human Colon Tumors

- Share similar morphological features
- Share similar molecular alterations
  - Contain point mutations in *Kras* or *Ctnnb1*
  - Have gene expression alterations within Wnt, MAPK, and TGF-β signaling pathways as well as other relevant CRC genes
- AVNWLE-induced colon tumors in F344 rats share similar morphological and molecular features with human colon cancer

*Pandiri et al., Toxicol Pathol. 2011;39(7):1065-74.*
**Mutational Analysis of Co-induced Rodent Lung Tumors**

<table>
<thead>
<tr>
<th>Cobalt metal dust (mg/m³)</th>
<th><strong>Kras mutation incidence (%)</strong></th>
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<tbody>
<tr>
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<td>B6C3F1/N mouse</td>
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<td>0</td>
<td>0/10 (0)*</td>
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<td>1.25</td>
<td>11/16 (69)***</td>
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<td>2.5</td>
<td>11/23 (48)**</td>
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<td>5.0</td>
<td>24/30 (80)***</td>
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<td>CMD-treated combined</td>
<td>46/69 (67)***</td>
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*p<0.001 within the chamber controls: significant trend by the Cochran-Armitage test
**p<0.05, ***p<0.01, ****p<0.001 within the exposed groups: significantly different from the chamber controls by the Fisher’s exact test

- **Unique mutations in codon 12 of Kras gene**
  - **G to T transversions** in cobalt metal dust induced A/B carcinomas
  - **G to A transitions** in spontaneous A/B carcinomas from historical controls

- **Similar findings in cobalt sulfate heptahydrate induced alveolar/bronchiolar tumors**

- **G to T transversions related to oxidative stress**

_Hong et al., Toxicol Pathol. 2015;43(6):872-82._
Cobalt Sulfate-induced ROS Production

A549 = transformed type II cells from human lung cancer
BEAS-2B = immortalized human bronchial epithelial cells

Control

CSH 10mM

Dichlorodihydrofluorescein Diacetate (DCFDA) Assay

Immuno-Spin trapping (IST) assay

DCFDA Assay: General oxidative activity

DHE Assay: Hydroxyl and superoxide radicals

IST: Trapping of radicals with 5,5-dimethyl-1-pyrroline N-oxide (DMPO)
• A recap on molecular studies in rodent tumors in the NTP

• **Goals of genomic studies on rodent tumors from NTP studies**

• Progress on genomic studies on mouse hepatocellular carcinomas from NTP studies

• NTP collaborations

• Potential future studies and collaborations
Technology Driving Science

• Sanger sequencing
  – Mutational hotspots (single gene/exon/codon level interrogation)
  – Mutations unique to cancer type or etiology (Curtiss and Vogelstein)

• Next generation sequencing
  – Whole genome or whole exome (all coding and non-coding regions)
  – Discovery of novel genetic/epigenetic events
  – Targeted sequencing of gene panels for screening

• Mutation signatures (Alexandrov, Stratton et al., 2013)
  – 6 types of substitutions: C>A, C>G, C>T, T>A, T>C, and T>G (all substitutions are referred to by the pyrimidine of the mutated Watson–Crick base pair)
  – 6 types of substitutions * 4 types of 5’ base * 4 types of 3’ base = 96 possible mutation types
Mutation Signatures (Alexandrov et al., 2013)

4 types of 5’ base * 6 types of substitutions * 4 types of 3’ base = 96
Mutational Signatures to Understand Mechanisms

**MUTATIONAL PROCESSES**
- DNA replication
- Genotoxins
- DNA repair deficiency
- DNA editing

**SOMATIC MUTATIONS**
- Small mutations
- Structural Variants
- Copy Number Variants
  - SNV
  - Indels
  - Translocation
  - Inversion
  - Loss
  - LOH
  - Gain

**MUTATIONAL SIGNATURES**
- Tumor sequencing
- Variant calling
- Genomic landscape
- Catalogue mutations
- Patterns of mutations
- Modeling
- Link patterns to mutagenesis

*Mylinhthibodeau, Wiki Commons*
## Environmental Exposures & Mutation Signatures

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<tr>
<th>Mut. Sig.</th>
<th>Exposure</th>
<th>Etiology</th>
<th>Characteristic DNA lesion</th>
<th>Signature hallmark</th>
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<td>1</td>
<td>N/A</td>
<td>Age</td>
<td>Spontaneous deamination of 5-methylcytosine, correlates with age</td>
<td>• C &gt; T</td>
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</tbody>
</table>
| 4         | Tobacco smoke  | (+)benzo(a)pyrene | (+)benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide-dG adduct                                  | • C > A  
• CC to AA tandem mutations  
• Transcriptional strand bias |
| 7         | Sunlight       | UV light          | Pyrimidine dimers                                                                          | • C to T at dipyrimidines  
• CC > TT tandem mutations  
• Transcriptional strand bias |
| 11        | Chemotherapy   | Temozolamide      | O6-methylguanine                                                                           | • C > T  
• Transcriptional strand bias |
| 22        | Food contaminant| Aristolochic acid| 7-(deoxyadenosin-N(6)-yl) aristolactam I adduct                                             | • T > A  
• Transcriptional strand bias |
| 24        | Food contaminant| Aflatoxin B1      | 8,9-dihydro-8-(N7-guanyl)-9-hydroxyaflatoxin B1 adduct                                    | • C > A  
• Transcriptional strand bias |
| 29        | Tobacco chewing| Mixtures          | Unspecified                                                                               | • C > A  
• CC to AA tandem mutations  
• Transcriptional strand bias |

[http://cancer.sanger.ac.uk/cosmic/signatures](http://cancer.sanger.ac.uk/cosmic/signatures), Hollenstein et al., Oncogene (2017) 36, 158–167
Mutational Signatures across Human Cancer

http://cancer.sanger.ac.uk/cosmic/signatures
Mutational Signatures across Human Cancer

http://cancer.sanger.ac.uk/cosmic/signatures
Mutational Signatures in Human Liver Tumors

http://cancer.sanger.ac.uk/cosmic/signatures

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Goals of Sequencing NTP Rodent Tumors

• Identification of mutation signatures in rodent tumors from defined exposures
  – Link the signatures to mechanisms of carcinogenicity
  – Potentially link human tumors to environmental exposures
  – Discovery of potential new mutation signatures

• Distinguishing spontaneous tumors from chemically induced tumors
  – Histologically indistinguishable
  – May provide a context/support for NTP’s carcinogenicity calls

• Identification of biomarkers for prediction of carcinogenicity from shorter-term *in vivo* studies or *in vitro* studies
  – Genomic and epigenomic approaches
  – Development of potential in vitro approaches
Overview

- A recap on molecular studies in rodent tumors in the NTP
- Goals of genomic studies on rodent tumors from NTP studies
- **Progress on genomic studies on mouse hepatocellular carcinomas from NTP studies**
- NTP collaborations
- Potential future studies and collaborations
Whole Exome Sequencing of Mouse HCC

An update..

Samples
• Spontaneous HCC (M, F), n=40
• Genotoxic chemicals (Gtx), n=40
• Non-genotoxic chemicals (NGtx), n=60
• Non-tumor controls (M, F), n=20

Methods
• Illumina exome paired-end sequence reads (150x)
• mm10 (alignment), B6C3F1/N, dbSNP, Mutect1

Results
• SNVs, mutation spectra, signatures, driver genes
Mutation Spectra of Mouse HCCs

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Somatic Signatures: NMF - Barchart

Mutation Signatures in Mouse HCCs

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- **S2**
- **S3**
- **S4**
- **S5**

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Next Steps for the Mouse HCC Project

- Examine mutation signatures in mitochondrial genomes and nuclear genomes from tumors
- High depth RNA-Seq to correlate with the exome data
- Copy number alterations using aCGH array
- mi-RNA Seq
- Whole genome bisulfite sequencing (David Adams - Sanger institute)
Overview

• A recap on molecular studies in rodent tumors in the NTP

• Goals of genomic studies on rodent tumors from NTP studies

• Progress on genomic studies on mouse hepatocellular carcinomas from NTP studies

• **NTP collaborations**

• Potential future studies and collaborations
Cancer Research UK Grand Challenge Grant: *Cancer mutation signatures to identify unknown cancer etiologies*

- International partnerships led by Sir Mike Stratton from the Wellcome Trust Sanger Institute
- Identify mutation signatures from known carcinogens (~150)
- *To identify and characterize the biological processes underlying mutation signatures (Allan Balmain (UCSF) and David Adams (Sanger))*
- NTP support for the cancer mutation signature project
  - Identification of chemical carcinogens, sample selection, pathology review, DNA isolation, ~30 NTP studies
- Sanger contribution for the cancer mutation signature project
  - Generated whole genome sequence data on NTP parental strains (C57BL6/N, C3H/HeN) as well as the B6C3F1/N hybrid
Other NTP Collaborations

IARC, Lyon, France

• Jiri Zavadil and Magali Olivier (Molecular Mechanisms and Biomarkers Group, Mechanisms of Carcinogenesis Section)

• To complement the IARC cancer monographs and also to provide a translational context to the rodent cancer data

• Pathology review, sample selection, and shipment of tissue sections

Ramazzini Institute, Bologna, Italy

• Fiorella Belpoggi and Andrea Vornoli

• TrueSeq Custom Amplicon Assay (TSCA) based on top 25 mutated genes in human gliomas

• TSCA analysis of rat brain tumors to examine human relevance
Overview

• A recap on molecular studies in rodent tumors in the NTP
• Goals of genomic studies on rodent tumors from NTP studies
• Progress on genomic studies on mouse hepatocellular carcinomas from NTP studies
• NTP collaborations

• Potential future studies and collaborations
Potential Future Studies/Collaborations

- Cancer mutation signatures from genotoxic chemicals are fairly strong and probably are conserved across species.
- Cancer mutation signatures from non-genotoxic chemicals may be variable due to multiple modes of action (MOA):
  - Activation of multiple nuclear receptors
  - Species and organ specificity
- Majority of the chemical carcinogens have a non-genotoxic mode of action, often with multiple MOAs.
- Generate multi-omics data from rodent tumor tissues resulting from exposures with a well defined *single* MOA:
  - Tumors derived from exposures that target specific nuclear receptors such as AhR, PPAR-α, CAR/PXR, etc.
  - Link each of the resulting mutation signature to a specific MOA.
Conclusion and future directions

**Human**

- Rodent Models
  - Conerved Molecular Pathways
    - Human relevance

- **Mechanistic understanding**
  - Integrated -omics approaches
    - Molecular pathways
    - Exposure
    - Neoplasia

- **Prediction**
  - Short-term *in vivo* screens
    - Epigenetic landmarks
    - Mutation signatures
    - Driver mutations
    - Gene expression
  - *in vitro* screens
    - Immortalized cells
    - 3D cell culture

Adapted from The Cancer Genome Atlas Research Network, 2017
Translational Toxicology Pipeline

Molecular Pathology: Phenotypic anchoring

- Mechanisms, Translation, Prediction
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• Allan Balmain (UCSF)
• Jiri Zavadil, Magali Olivier (IARC)
• Fiorella Belpoggi, Andrea Vornoli (RI)

Epigenomic core

• Greg Solomon

Microarray core

• Kevin Gerrish

Bioinformatics core

• Ashley Brooks
• Adam Burkholder
• Pierre Bushel
• Jianying Li
• Jason Li