NTP
National Toxicology Program

Overview of Genomic Studies on Rodent Tumors and Its Translational Relevance

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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

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Rodent Models Provide a Translational Perspective

Comparisons of Leading Sites of New Cancer Cases in Humans to Tumor Sites in Animal Models

♂		♀	
Lung	Lung & Bronchus	Lung & Bronchus	Lung
Prostrate – Uncommon	Prostate	Breast	Mammary Gland
Intestine	Colon & Rectum	Colon & Rectum	Intestine
Pancreas	Pancreas	Pancreas	Pancreas
Liver	Liver	Ovary	Ovary
Lymphoma	Non-Hodgkin's Lymphoma	Non-Hodgkin's Lymphoma	Lymphoma
Lymphoma	Leukemia	Leukemia	Lymphoma
Esophagus – Uncommon	Esophagus	Uterine Corpus	Uterus
Urinary Bladder	Urinary Bladder	Brain	Brain
Kidney	Kidney	Liver	Liver

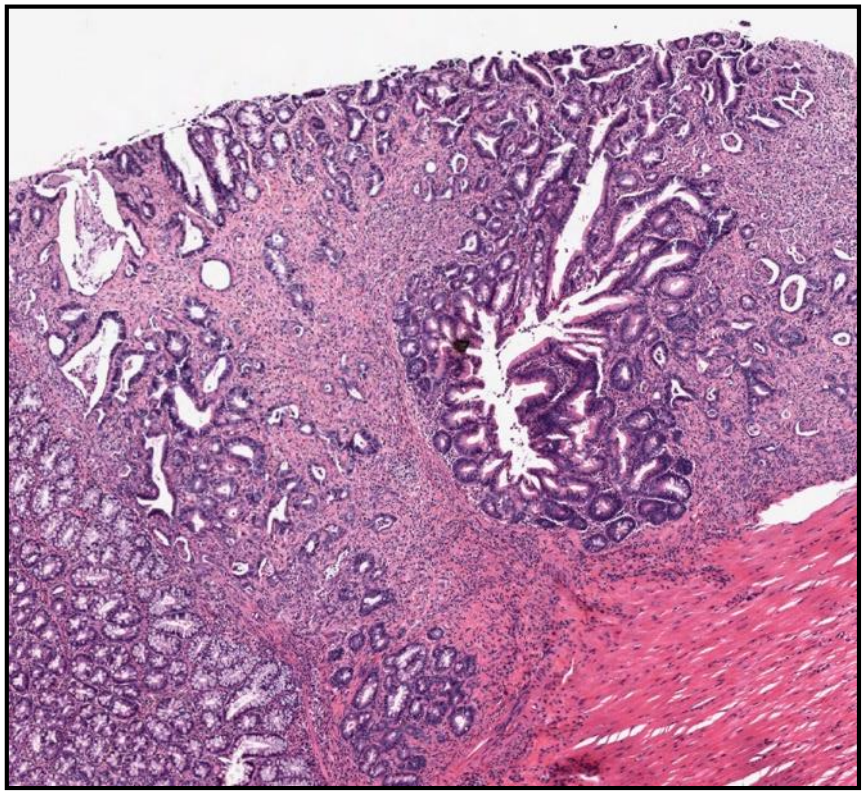
Molecular Studies on NTP Rodent Tumors - A Recap

- 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 *Kras* mutations in mouse lung tumors that are distinct from mutations in 1,3-butadiene exposures (Sills *et al*., 1999)
  - *Kras*, *Egfr* and *Tp53* mutations in mouse and rat lung tumors from cobalt metal and cobalt sulfate heptahydrate exposures (Hong *et al*., 2015)
  - Alterations in MAPK, WNT, and TGF-β signaling in large intestinal tumors in rats exposed to Aloe vera extract (Pandiri *et al*., 2011)

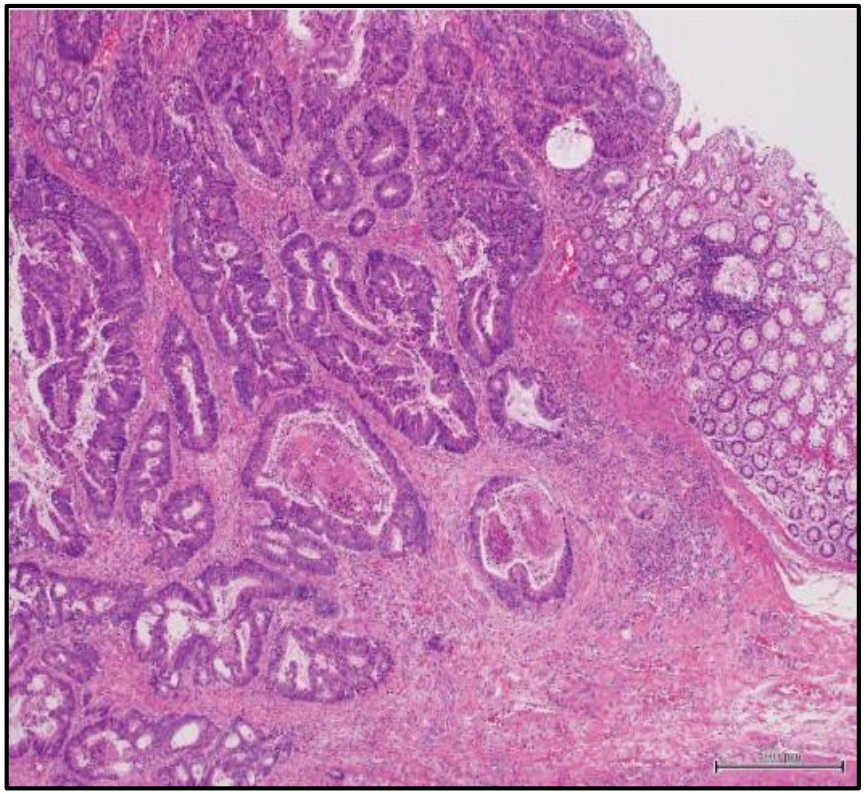
Histopathology of the large intestinal tumors in rats exposed to AVNWLE

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

Colon adenocarcinoma - Rat

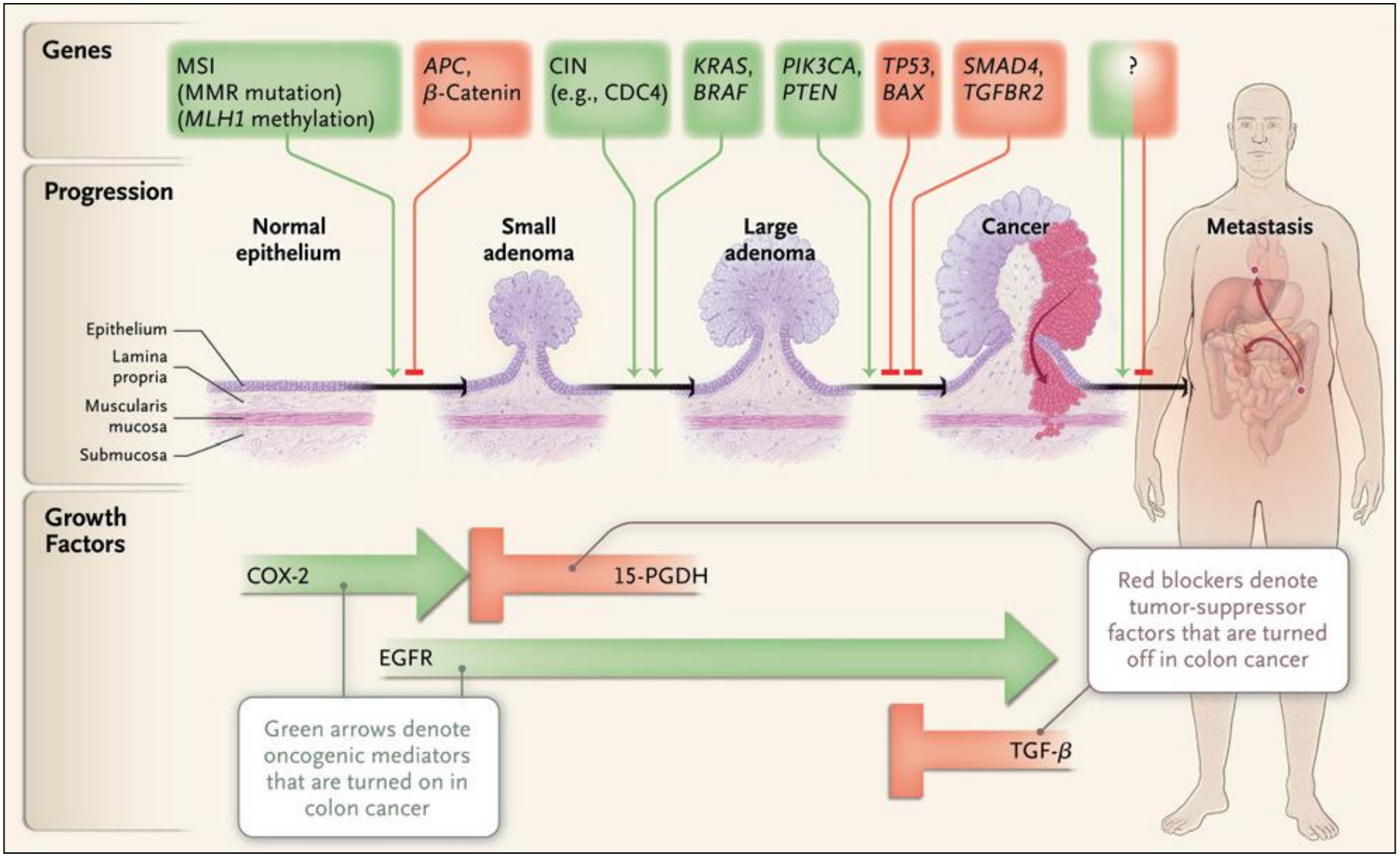


Colon adenocarcinoma - Human



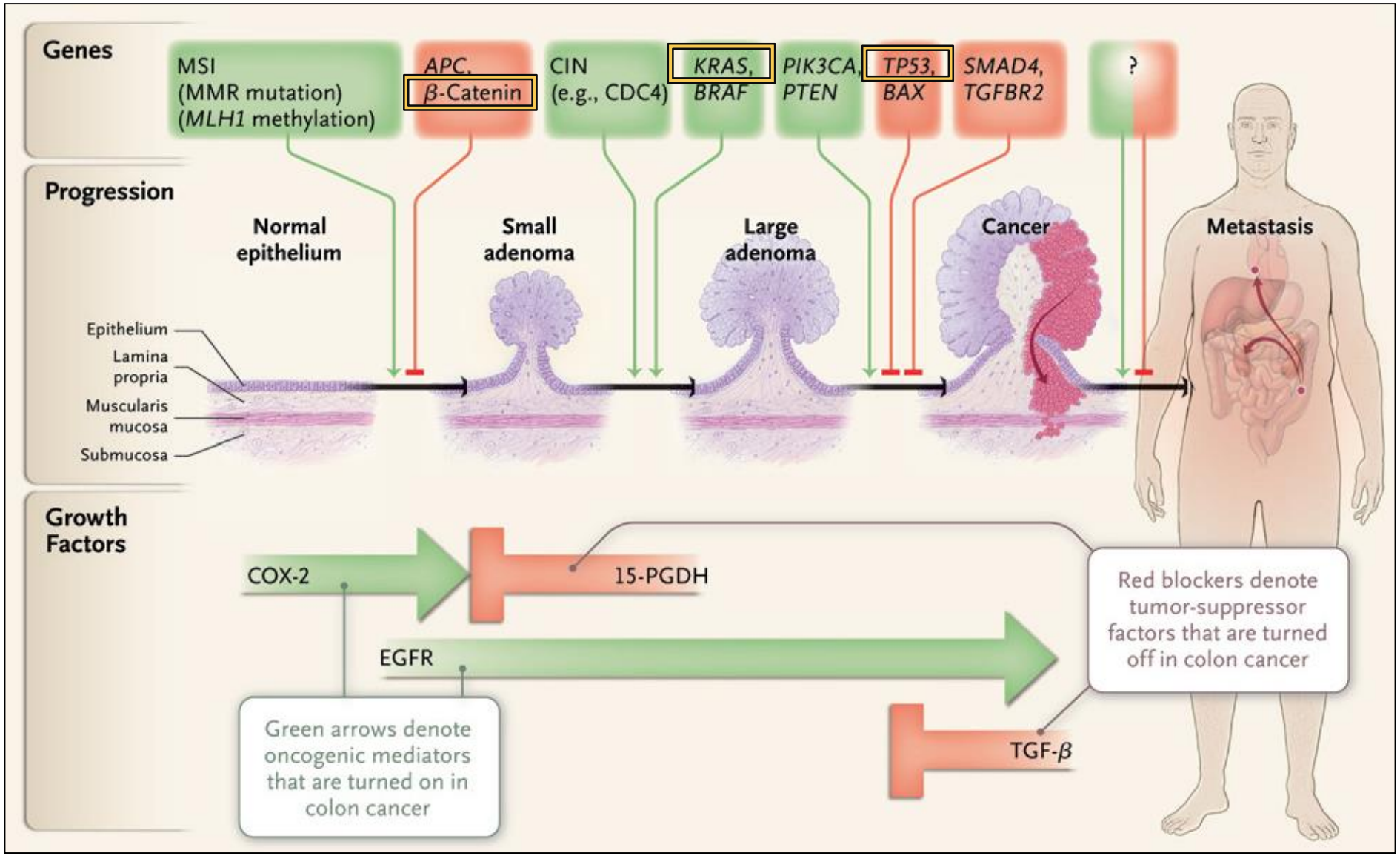
Hisatsune et al., 2013

Molecular Alterations in Human Colorectal Cancer



Sanford et al., N Engl J Med 2009;361:2449-60.

Molecular Alterations in Human Colorectal Cancer



Sanford et al., N Engl J Med 2009;361:2449-60.

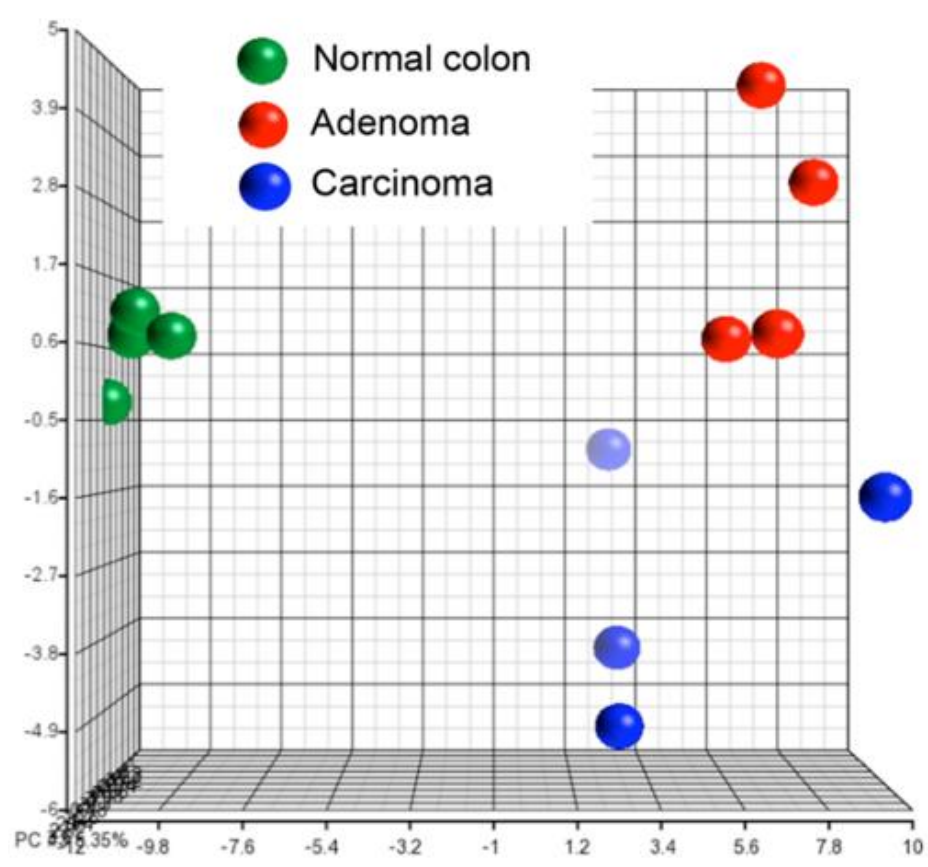
Mutation Analysis using Sanger Sequencing

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

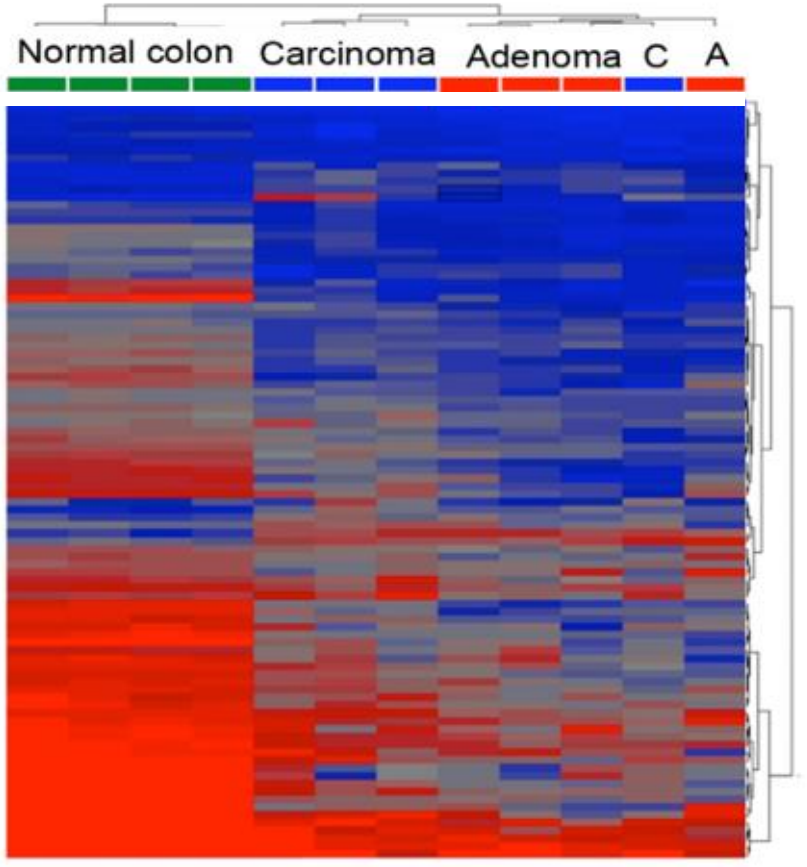
Group	% *Ctnnb1* mutations	% *Kras* mutations	% *Tp53* mutations
AVNWLE	33	33	0
Human CRC	15-26	40-60	50*
Azoxymethane	50-80	30-60	0
Heterocyclic Amines	50-75	0-14	0

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

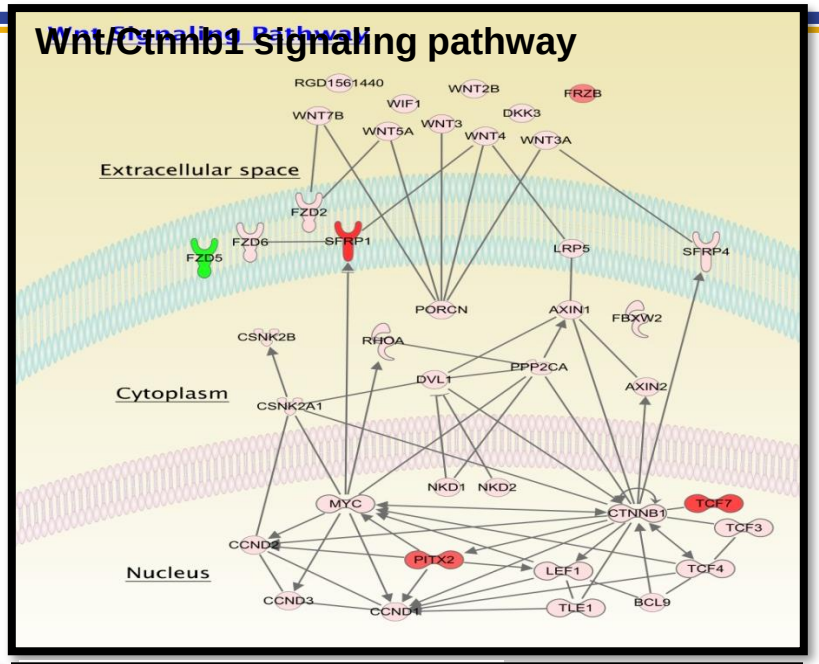


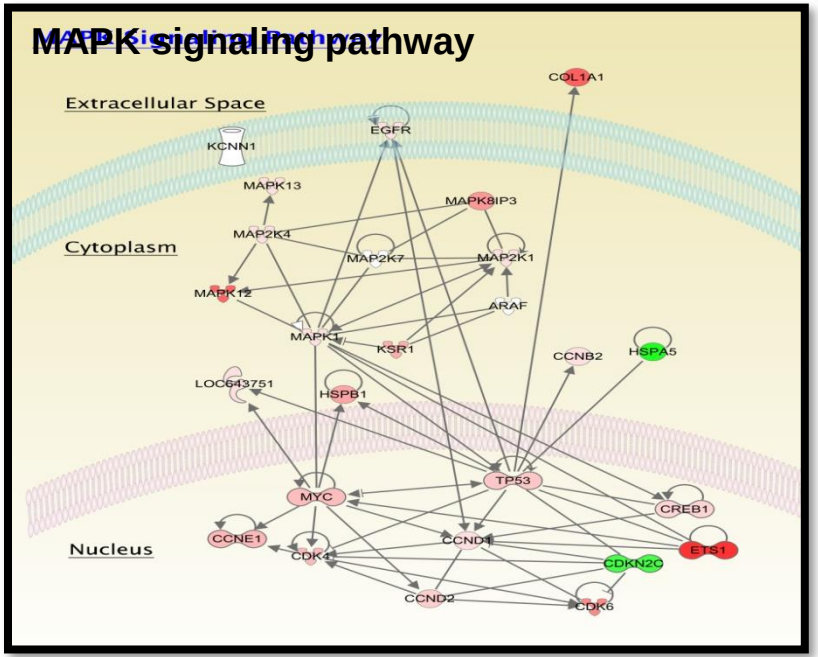
Hierarchical Cluster Analysis

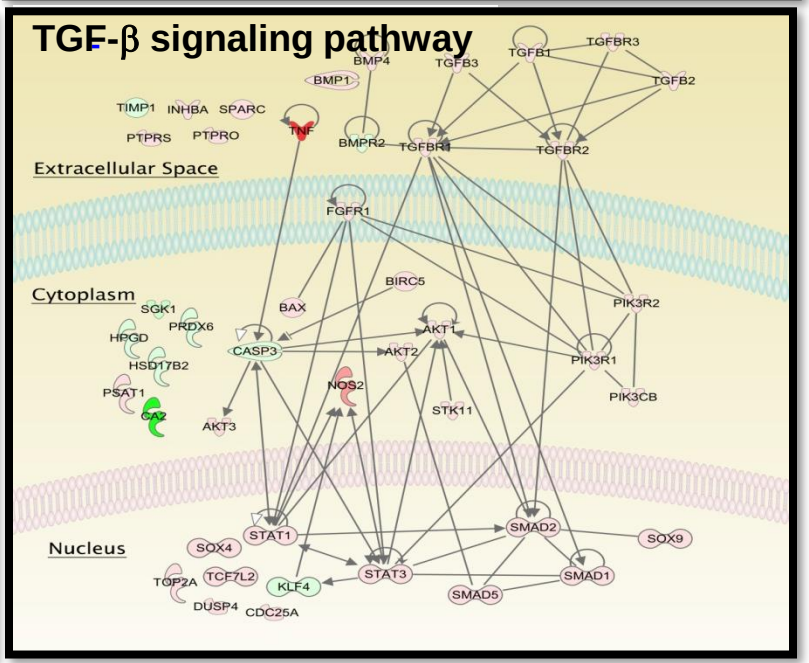
Molecular Alterations in AVNWLE-induced Large Intestinal Tumors

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







Summary

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

Cobalt metal dust (mg/m^3)	*Kras* mutation incidence (%)	
	B6C3F1/N mouse	F344/NTac rat
0	0/10 (0)*	0/10 (0)**
1.25	11/16 (69)***	2/14 (14)
2.5	11/23 (48)**	6/17 (35)*
5.0	24/30 (80)***	7/17 (41)*
CMD-treated combined	46/69 (67)***	15/48 (31)*

*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

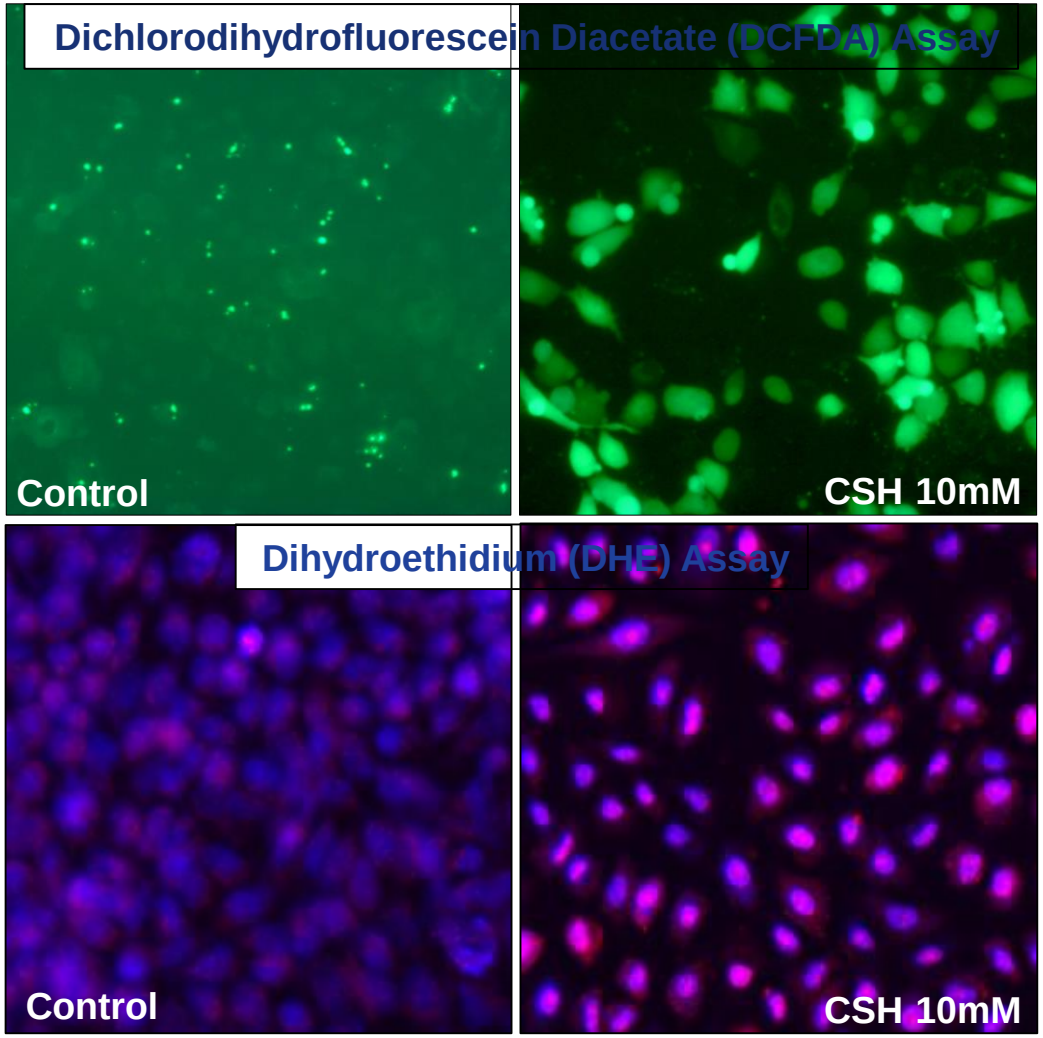
- Unique mutations in codon 12 of *Kras* gene
  - G to T transversions in cobalt metal dust induced A/B carcinomas
  - G to A transitions in spontaneous A/B carcinomas from historical controls
- Similar findings in cobalt sulfate heptahydrate induced alveolar/bronchiolar tumors
- G to T transversions related to oxidative stress

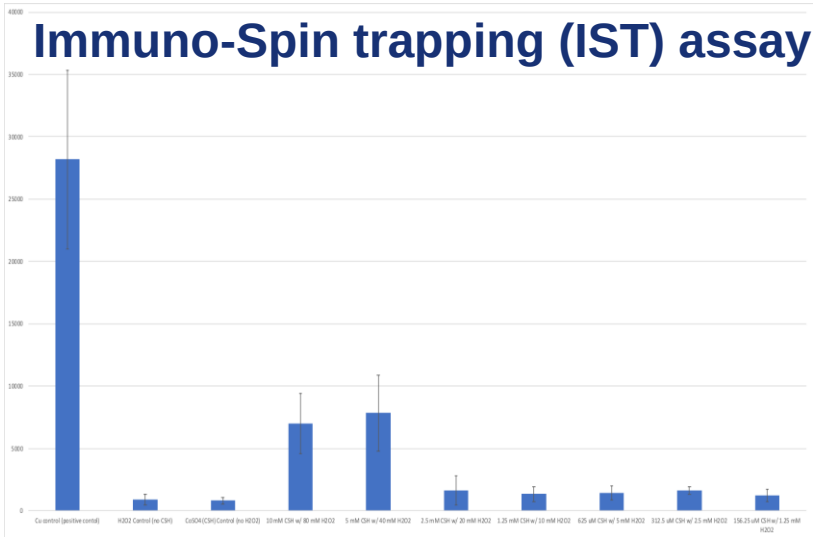
Hong et al., Toxicol Pathol. 2015;43(6):872-82.

Cobalt Sulfate-induced ROS Production

A549 = transformed type II cells from human lung cancer

BEAS-2B = immortalized human bronchial epithelial cells





DCFDA Assay: General oxidative activity

DHE Assay: Hydroxyl and superoxide radicals

IST: Trapping of radicals with 5,5-dimethyl-1-pyrroline N-oxide (DMPO)

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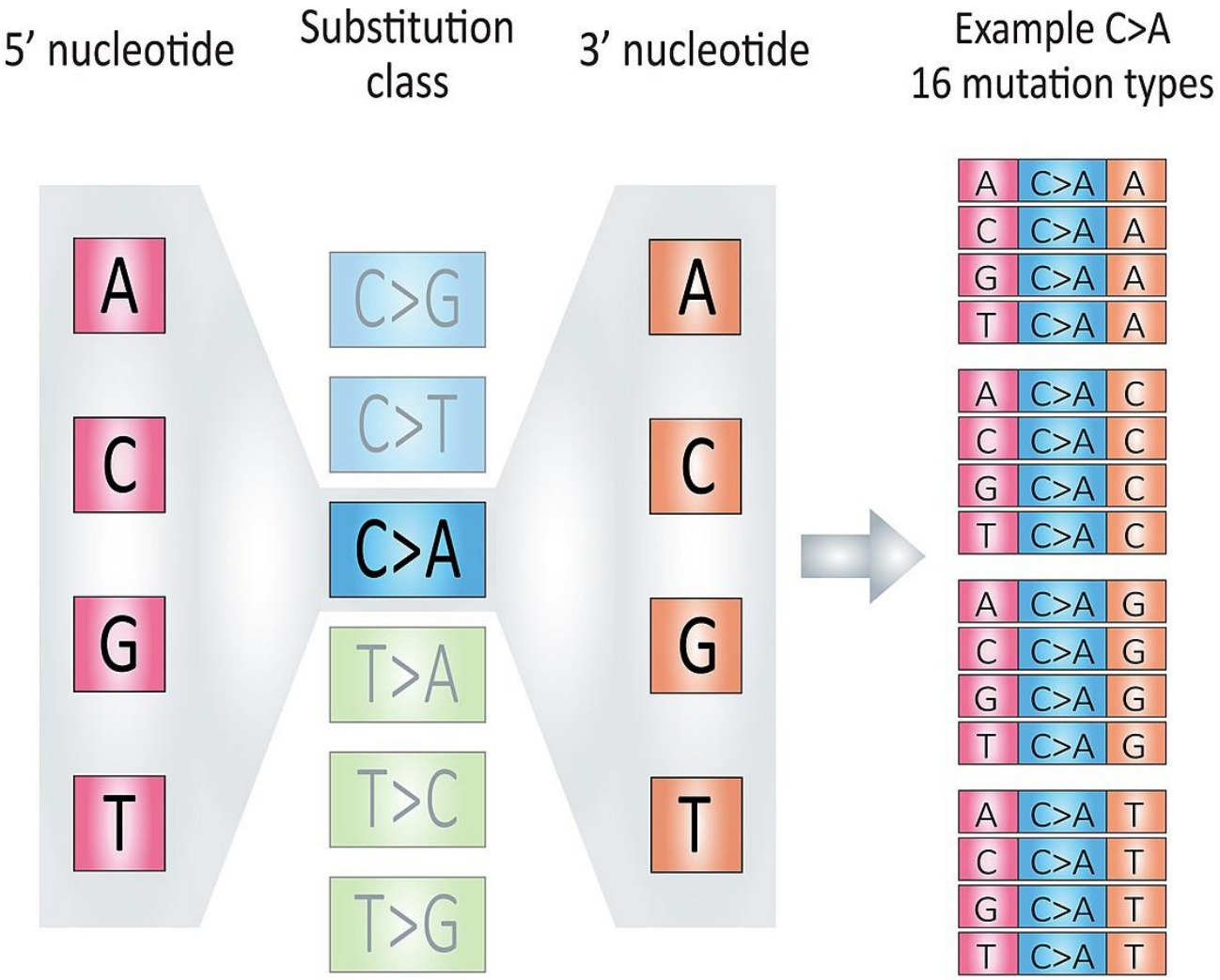
Technology Driving Science

- 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

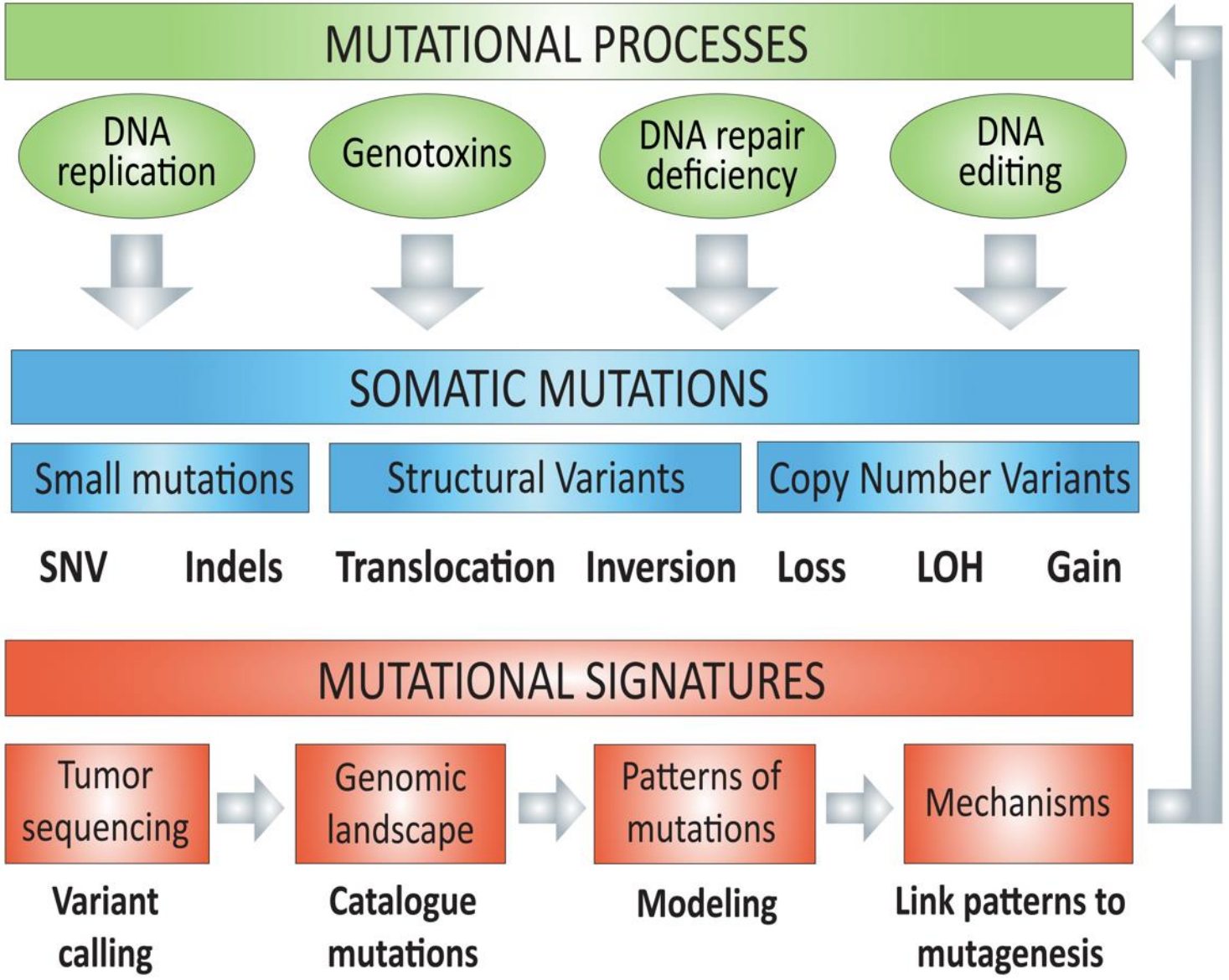
Mutation Signatures (Alexandrov et al., 2013)



4 types of 5' base * *6* types of substitutions * *4* types of 3' base = 96

Mylinhthibodeau, Wiki Commons

Mutational Signatures to Understand Mechanisms



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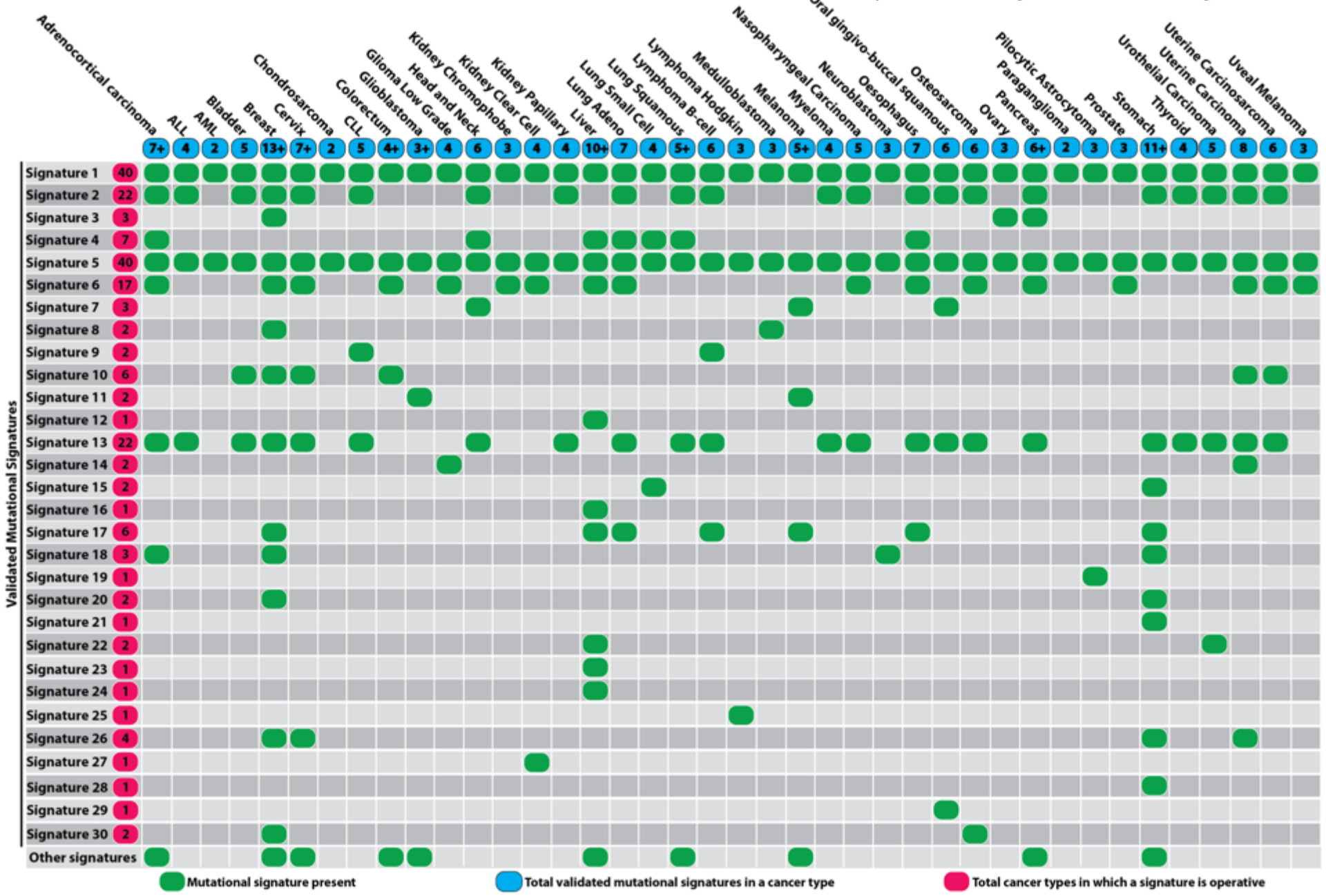
Environmental Exposures & Mutation Signatures

Mut. Sig.	Exposure	Etiology	Characteristic DNA lesion	Signature hallmark
1	**N/A**	Age	Spontaneous deamination of 5-methylcytosine, correlates with age	• C > T
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	O^6-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, Hollenstein et al., Oncogene (2017) 36, 158–167

Mutational Signatures across Human Cancer

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



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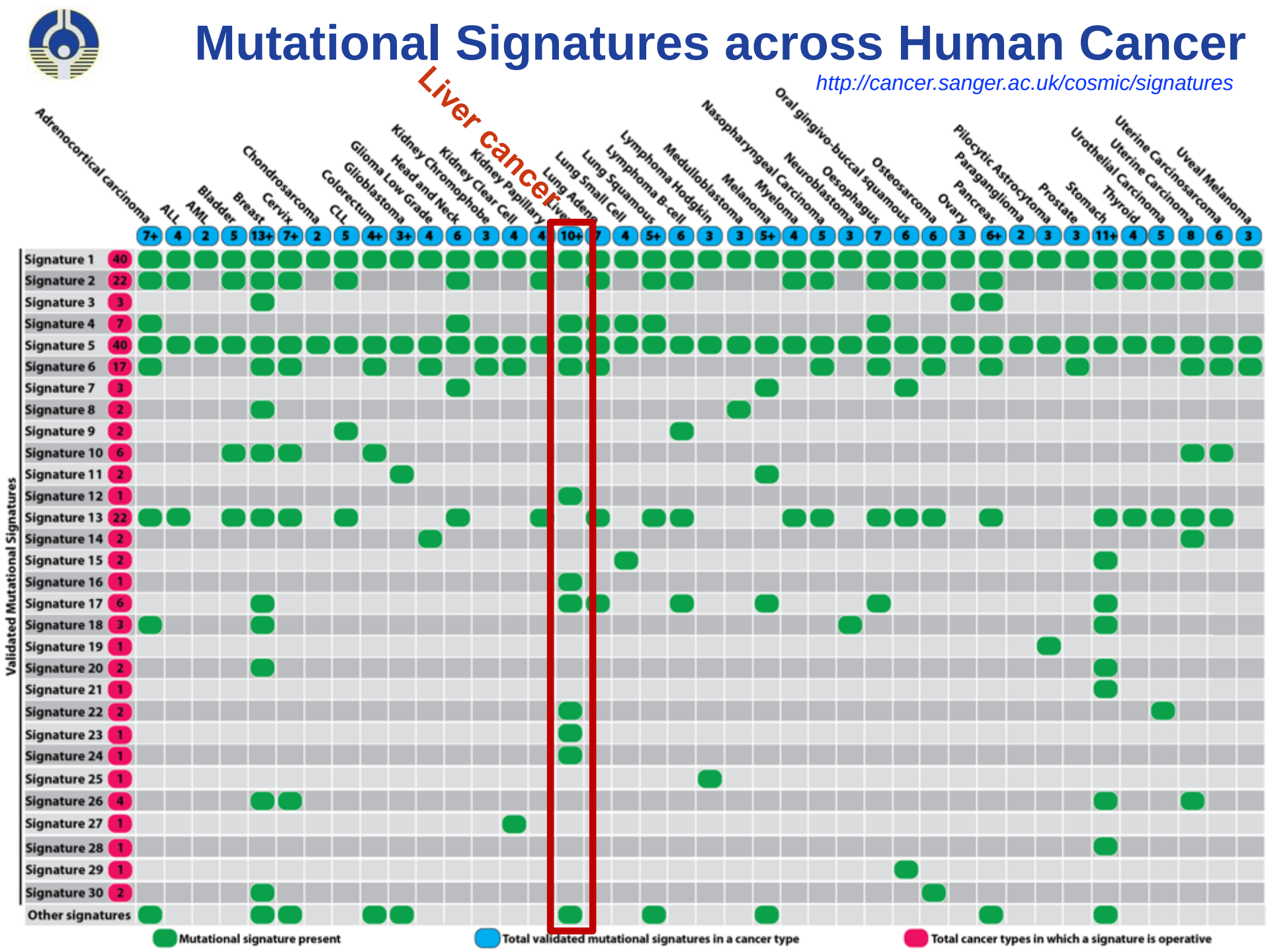
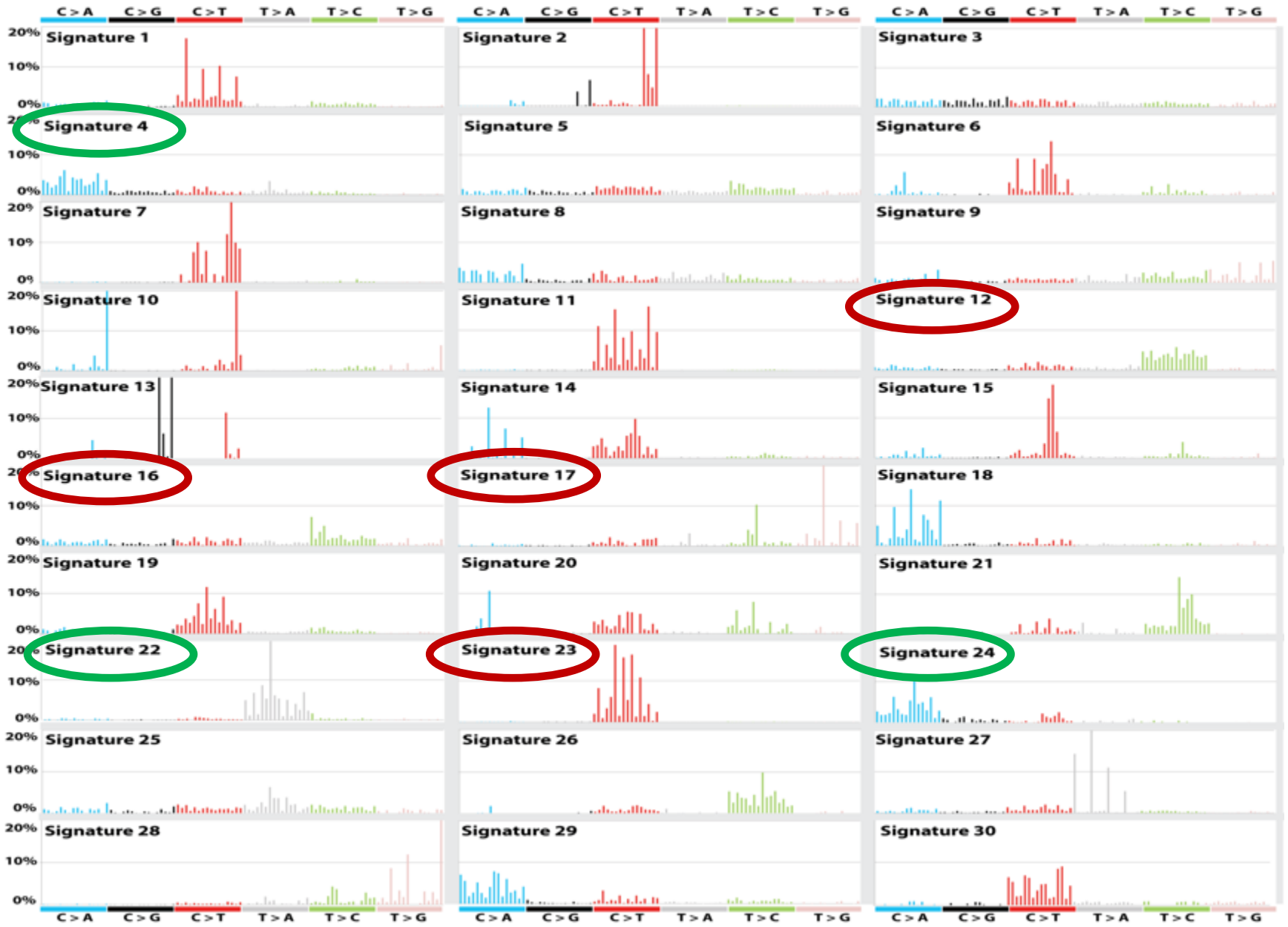


Mutational Signatures in Human Liver Tumors

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



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

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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*

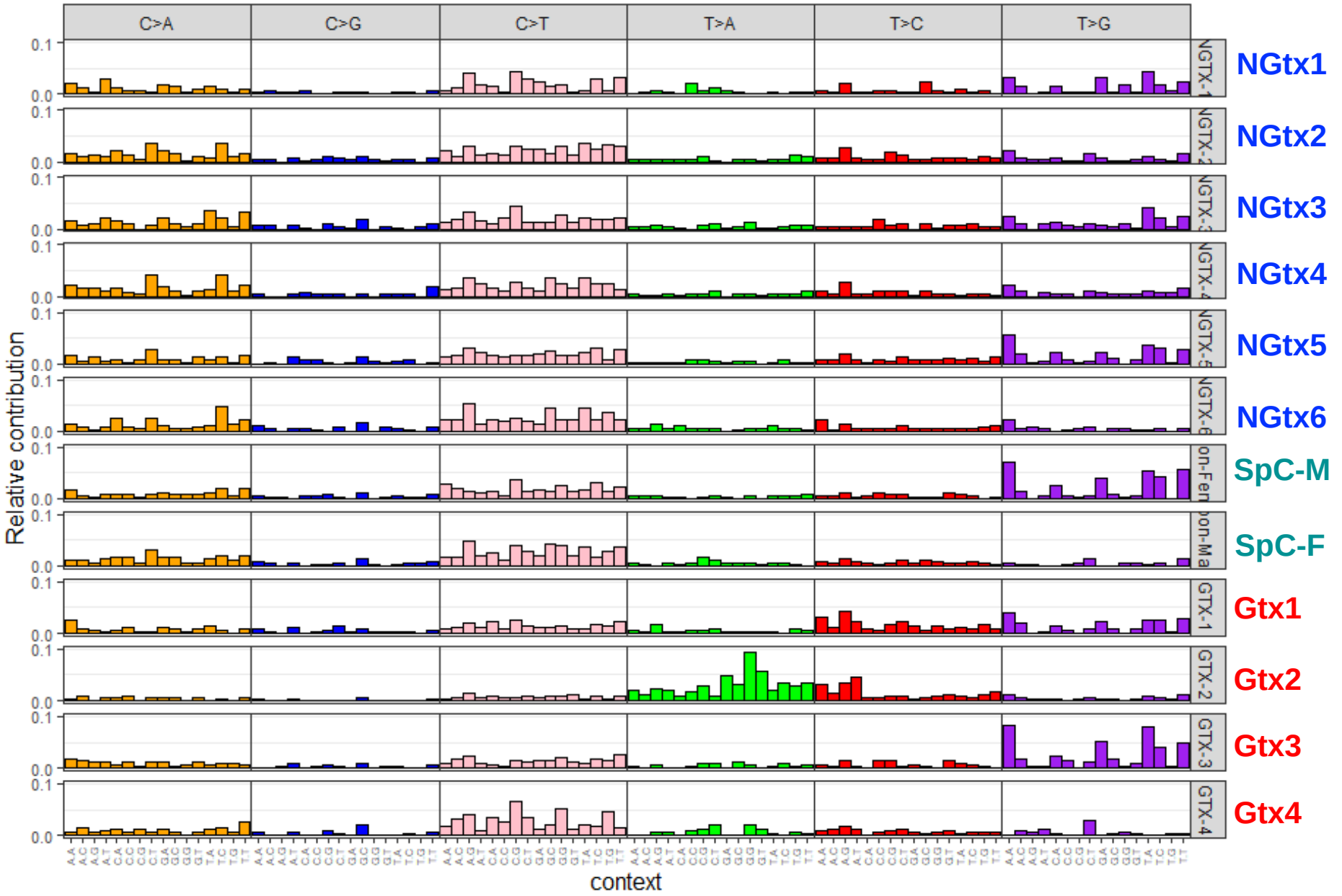
Methods

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

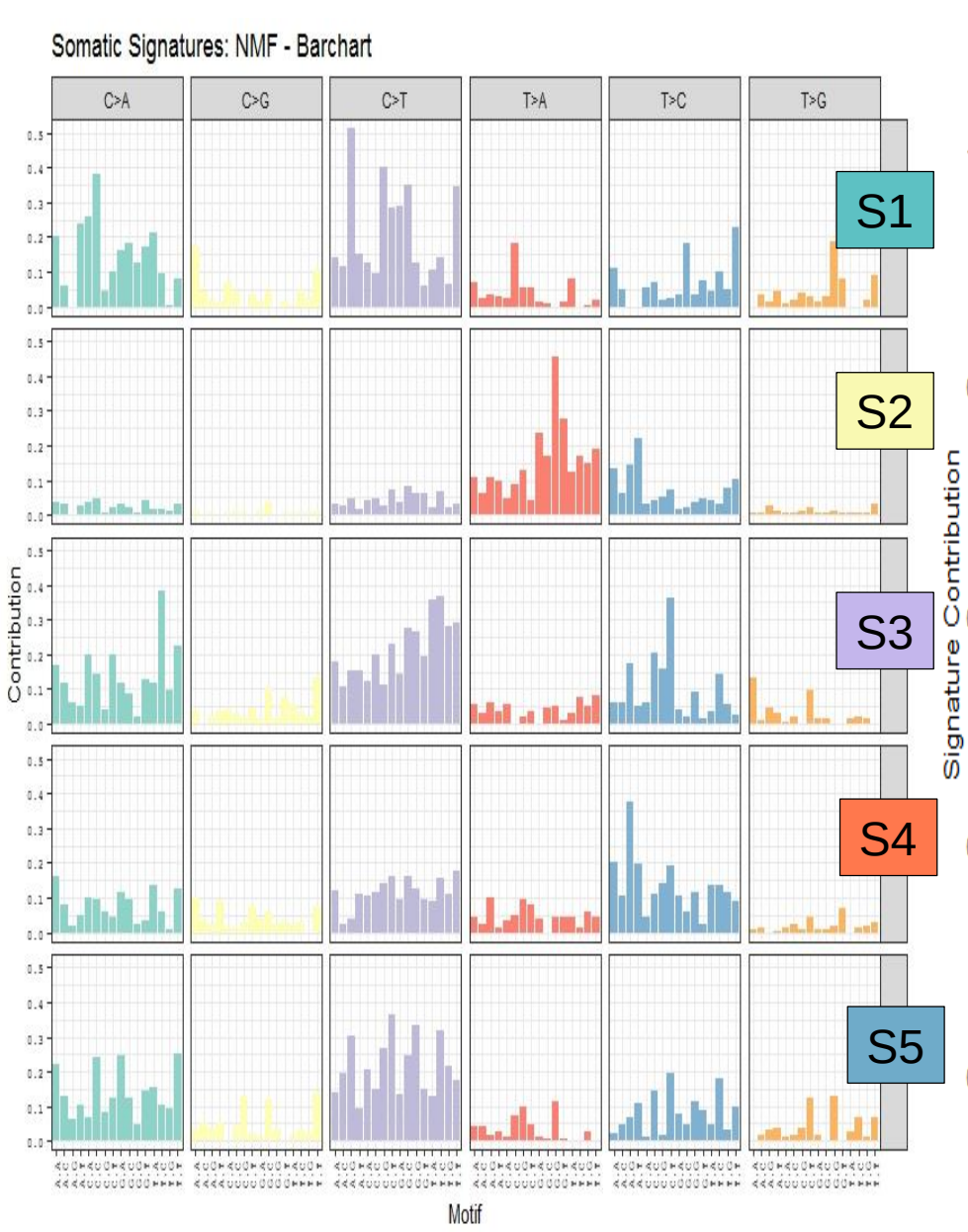
Results

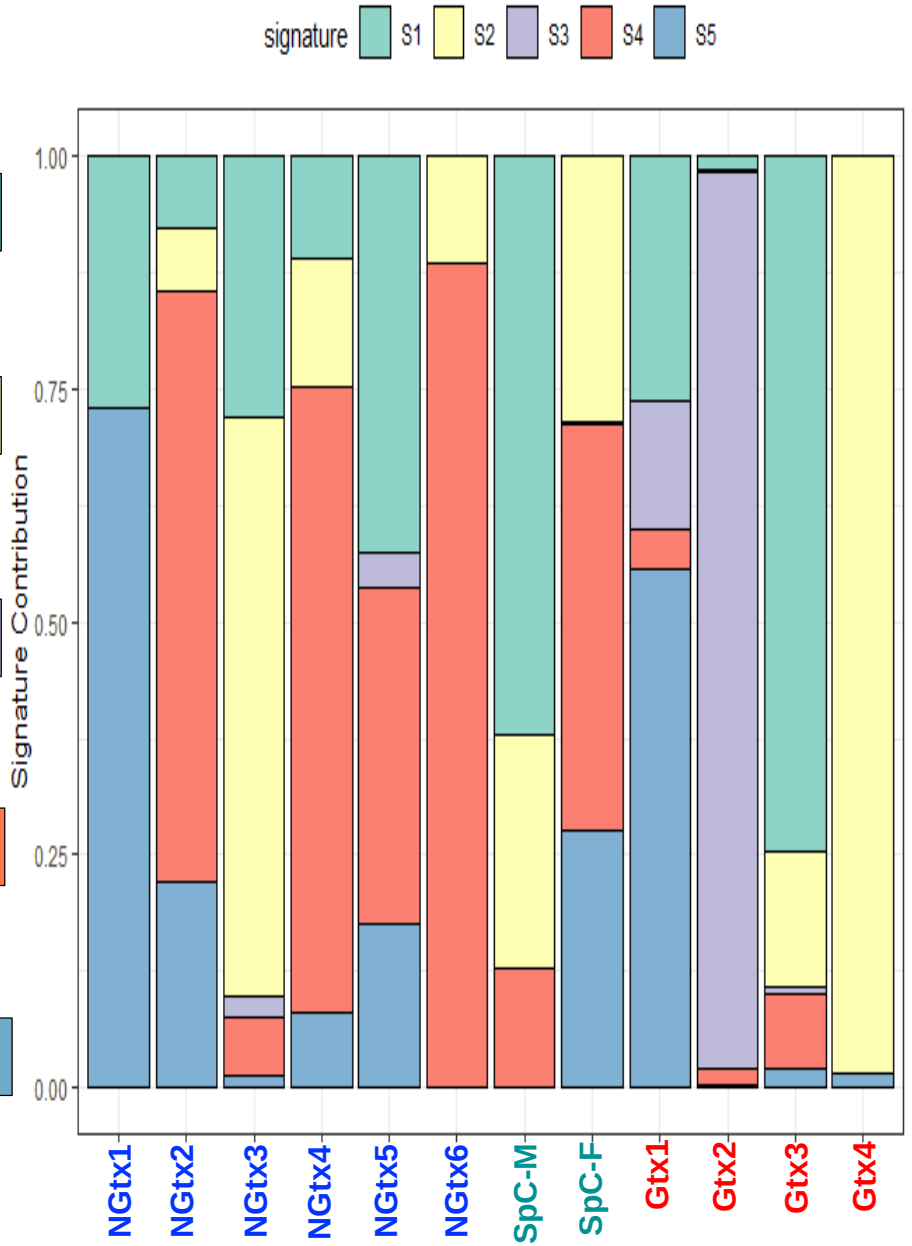
- SNVs, mutation spectra, signatures, driver genes

Mutation Spectra of Mouse HCCs



Mutation Signatures in Mouse HCCs





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)

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NTP, UCSF, and Sanger Collaboration

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

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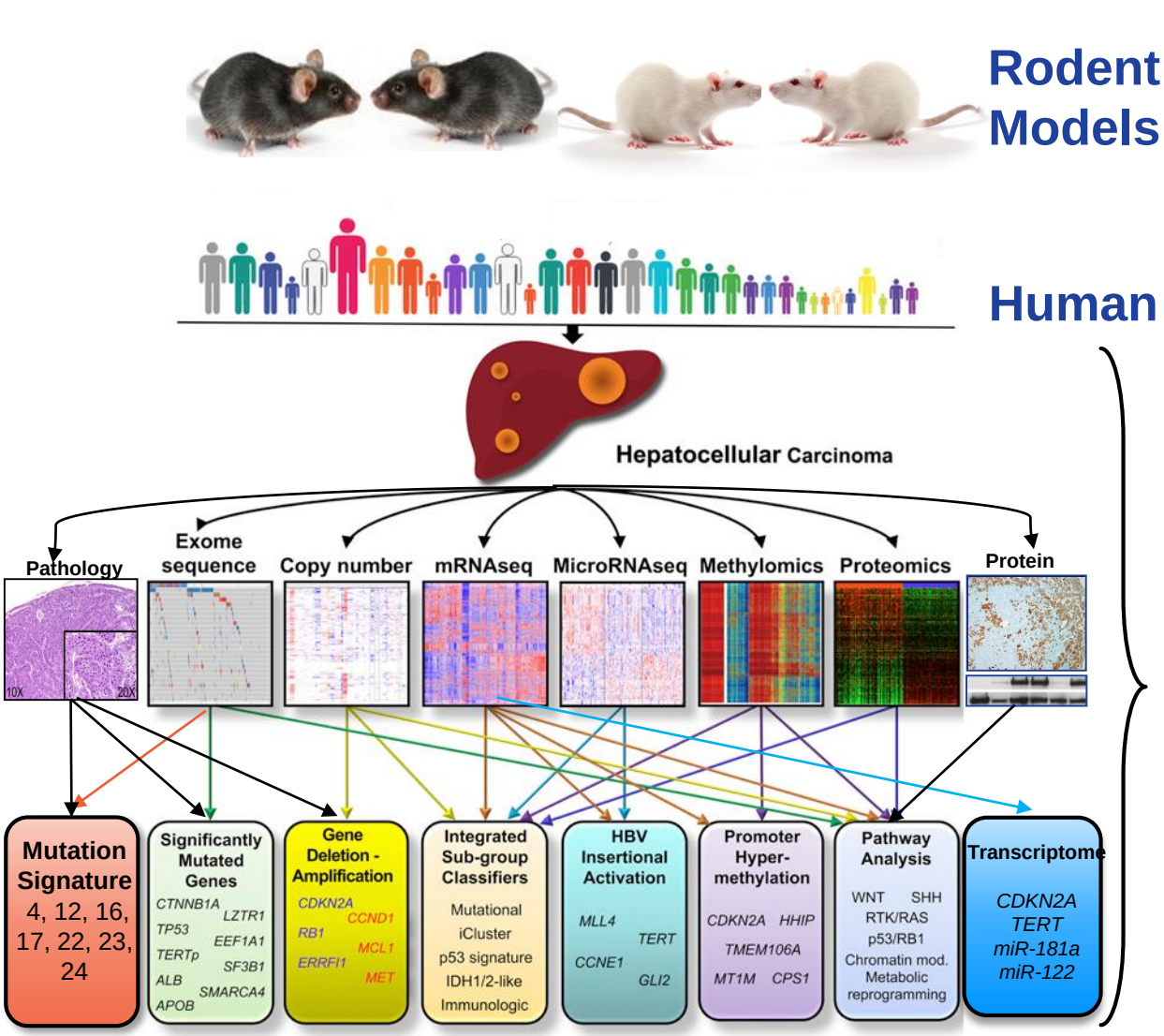
Potential Future Studies/Collaborations

- 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



Translation

Conserved Molecular Pathways

- Human relevance

Mechanistic understanding

Integrated -omics approaches

- Molecular pathways

Identify biomarkers

- Exposure
- Neoplasia

Prediction

Short-term *in vivo* screens

- Epigenetic landmarks
- Mutation signatures
- Driver mutations
- Gene expression

***in vitro* screens**

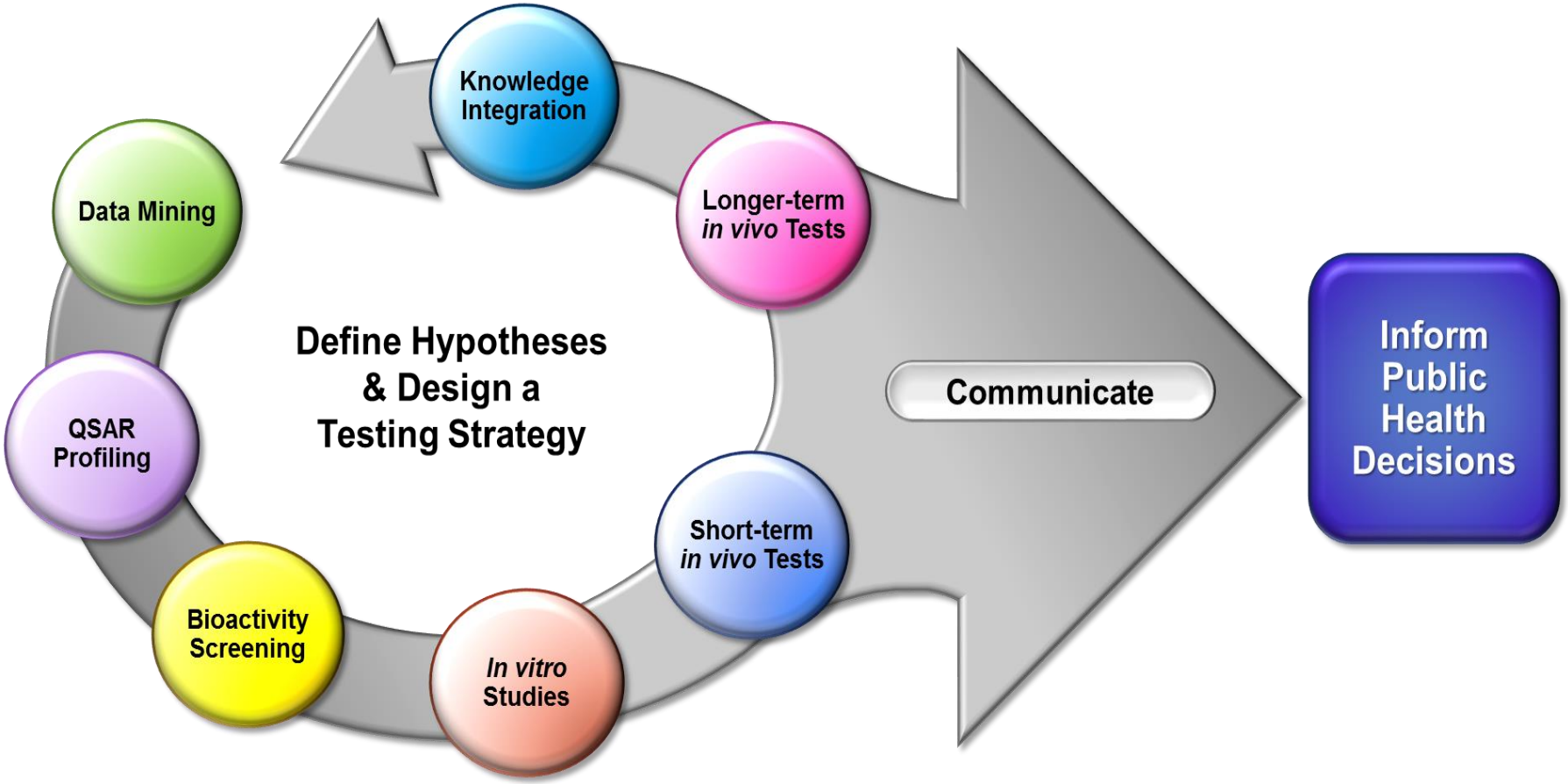
- Immortalized cells
- 3D cell culture

Adapted form 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