Application of 'Omics' Technologies to Assess Chemical Respiratory Allergy

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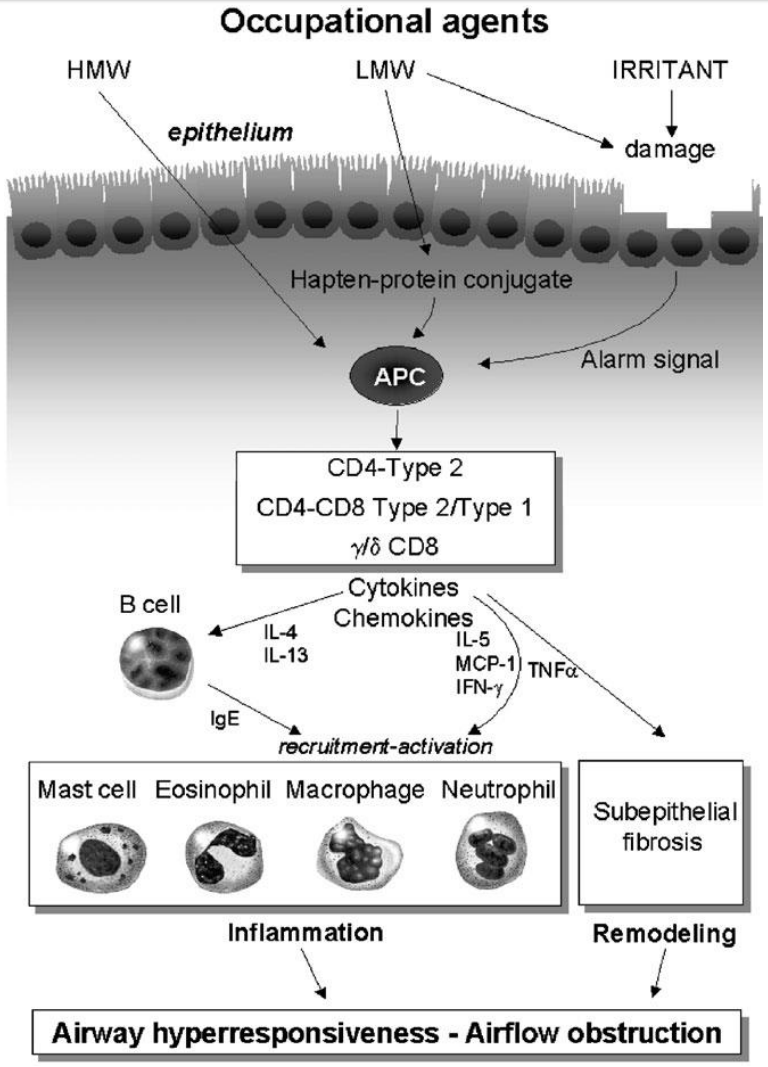
Overview

- Essential concepts
- Application of ‘omics’ in the characterization of respiratory sensitization/allergy
  - Elicitation models
    - Sensitization and challenge
  - Induction models
    - Leveraging the LLNA
- Why aren’t all chemical sensitizers Respiratory Sensitizers?
- Closing thoughts…

Essential Concepts

- Dermal Sensitizers ≠ Respiratory Sensitizers
  - Th1- vs Th2-biased response
  - Contact dermatitis vs allergic respiratory effects
- Sensitizers have thresholds of induction and elicitation and differing levels of potency
- WoE approach currently used to distinguish respiratory sensitizers from dermal sensitizers
- Toxicogenomics can provide an *unbiased* global assessment of gene-expression and protein network alterations
  - Hypothesis generating
  - Insights into MoA

Path to Respiratory Sensitization



- **Induction/Sensitization**
  - Initial molecular interactions
    - Hapten-protein, epithelial cells, PRRs
  - Dendritic Cell Activation
    - Initiating a Th2-bias
  - Lymphoid Cell Activation
    - Proliferation, Differentiation

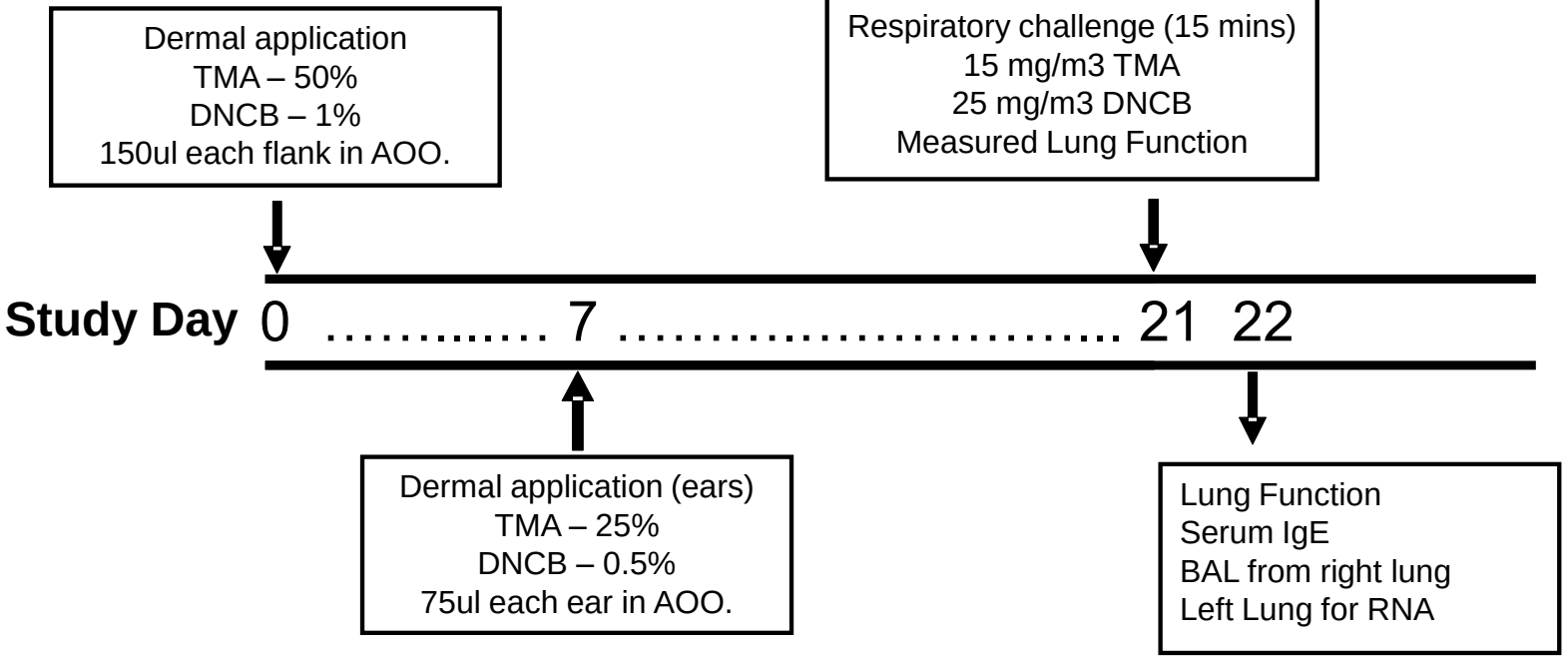
- **Elicitation**
  - Localization/Amplification of allergic response
  - Epithelial Remodeling
    - Effector/Inflammatory Cell Influx
    - Mucous Cell Hyperplasia/Metaplasia
  - Functional Pulmonary Responses
    - Airway hyperreactivity (AHR)
    - Reversible airflow obstruction

Can Genomics Enhance Identification of Respiratory Sensitizers?

- Two papers by Kuper et al.
  - **Molecular Characterization of Trimellitic Anhydride-induced Respiratory Allergy in Brown Norway Rats-** *Tox Path*, 36: 985-998, 2008
  - **The contact allergen dinitrochlorobenzene (DNCB) and respiratory allergy in the Th2-prone Brown Norway rat-** *Toxicology* 246 (2008) 213–221
- Improve hazard identification and cross-species comparisons (rodent to human) of respiratory allergens through molecular characterization
- Whole genome analysis performed and results related to physiological and cellular parameters
- Compared respiratory and dermal sensitizer responses in same model system

Do Respiratory and Dermal Sensitizer Induce Similar Responses?

- **Sensitization – Challenge Model**
  - Brown Norway Rat (BN)
  - Respiratory sensitizer = TMA; Dermal Sensitizer = DNCB
  - Experimental Groups
    - Non-sensitized / non challenged (-/-)
    - Sensitized / non-challenged (+/-)
    - Non-sensitized / challenged (-/+)
    - Sensitized / Challenged (+/+)



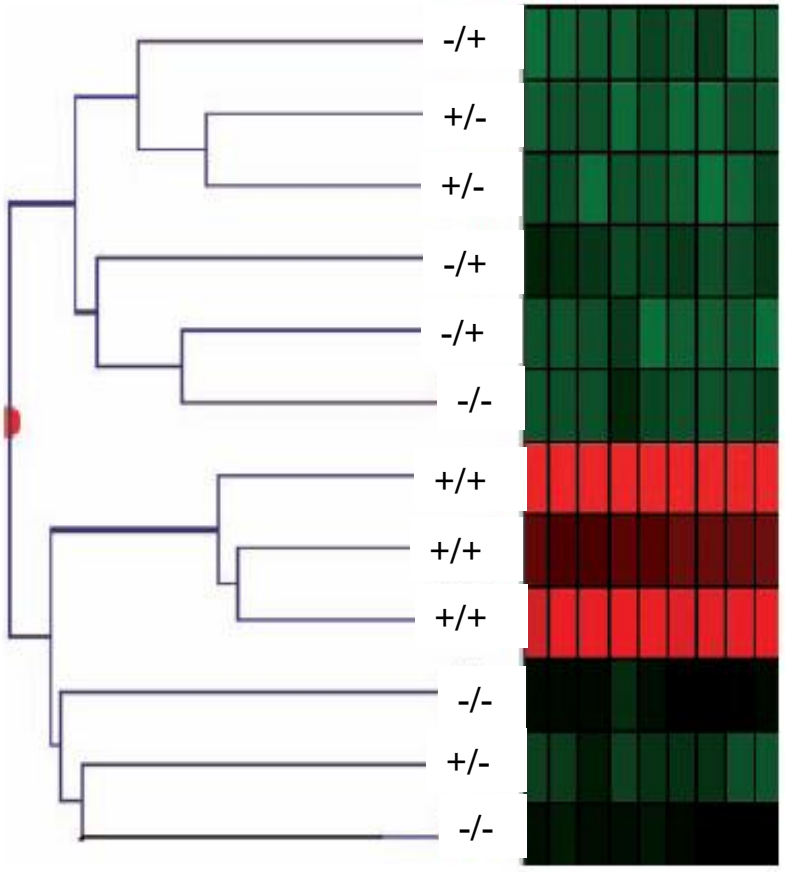
Respiratory and Dermal Sensitizers: Different Functional Responses

- Results- physiological and cellular endpoints

Endpoint	TMA	DNCB
Lung Function	Altered responses in +/+ only	No challenge-specific effects
Serum IgE	Increased in +/- and +/+ only	No increases in any group
BAL	Increased EOS in +/+ only	No effect
Lung immunohisto chemistry (IHC)	Increased IgE and CD4+ staining in +/+ only	Increased CD4+ in +/- and +/+ only

Respiratory and Dermal Sensitizers: Differences in Gene Expression

- **Microarray Results**
  - Clustering showed a clear separation between +/+ and the other groups for TMA
    - Not DNCB
  - No clear separation of -/-, +/-and -/+ for either TMA or DNCB
  - Gene groups/pathways ↑ by TMA
    - Chemokine activity, chemotaxis, inflammatory response, extracellular space/region, cytokine-cytokine receptor interaction, Toll-like receptor signaling pathway



Lung Remodeling Transcriptome Up-regulated Only by TMA

- Microarray Results- **DNCB vs TMA**

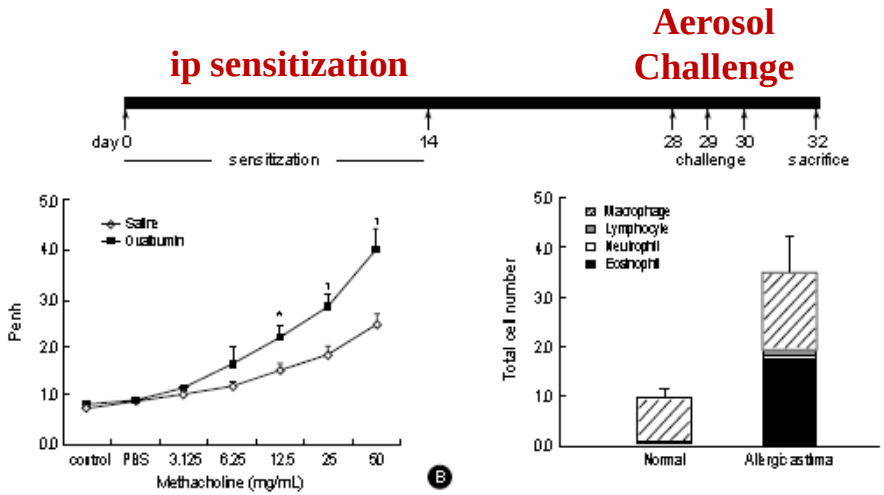
Gene	DNCB Fold change +/+ vs -/+	TMA Fold change +/+ vs -/+
Ccl2 (MCP1)	11	214
Ccl4 (MIP 1 beta)	5	267
Ccl7 (MCP3)	16	294
Ccl17 (TARC)	97	44
Arg1	NS	8
Timp1	NS	12
Il1b	NS	6
Il6	NS	38

- **Chemokine responses much greater in magnitude for TMA when compared to DNCB**
- **Lung remodeling genes were unique to TMA**

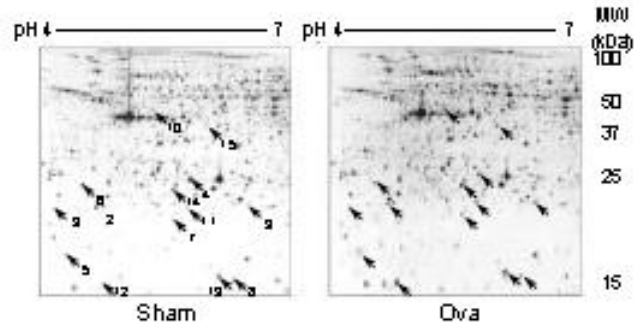
Genomics Show Promise to Differentiate Respiratory and Dermal Sensitizers

- Gene expression consistent with distinct physiologic and functional responses to TMA and DNCB
- Lung remodeling genes up-regulated in +/+ and -/+ TMA rats consistent with lung remodeling observed in early development of asthma in man
- Cytokines up-regulated in TMA +/+ BN rats are increased in sputum, BAL and exhaled air of human asthmatics
  - Toll-like receptor pathway activated in inflammatory conditions, like asthma, in man
  - Strongly up-regulated Arg-1 linked to Th2 cytokine expression and STAT6-dependent pathways
- Early lung remodeling may be a useful biomarker of respiratory sensitizers in animal models

Can Proteomics Enhance Identification of Respiratory Sensitizers?



(HoeSu Jeong, et al. J. Korean Med Sci 20:579-85, 2005)



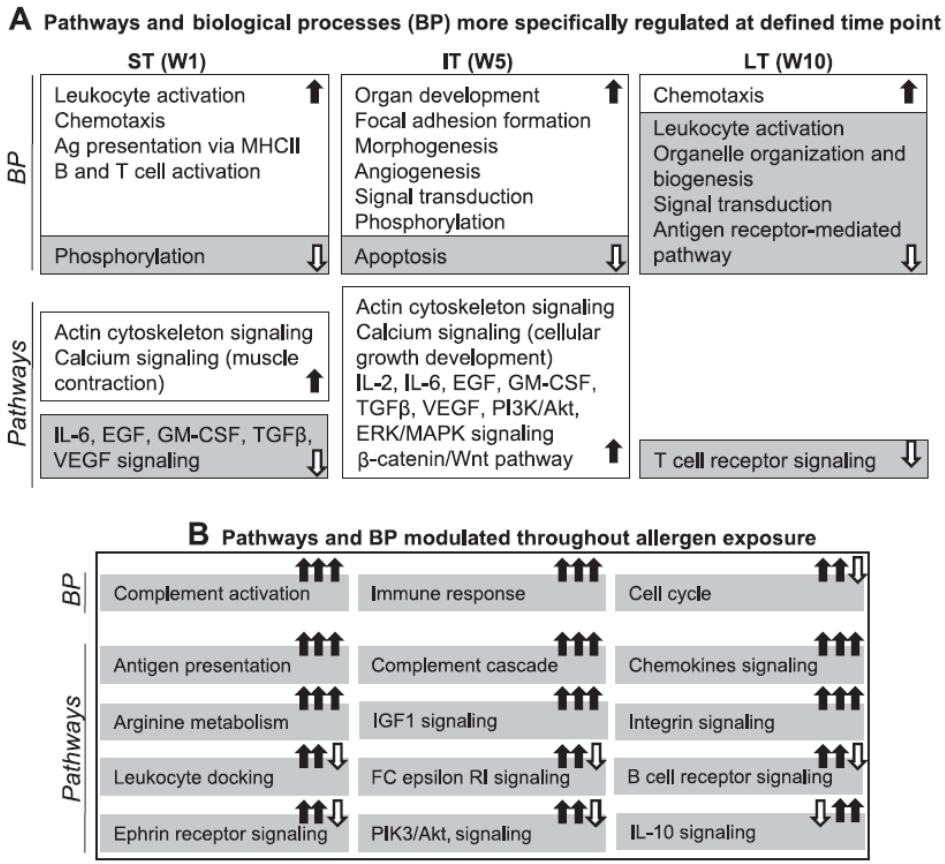
- Mouse model of allergic asthma
- Lung tissue proteins separated by 2DE
- Analyzed by MALDI-TOF MS
- 15 differentially expressed proteins
  - 5 were down-regulated in allergic mice
  - 8 were up-regulated in allergic mice
- 4 proteins associated with oxidation and reduction
  - Cytochrome b5, peroxiredoxins 1,2, and 6
- 3 classified as structural proteins (airway remodeling)
  - Rho-GDH dissociation inhibitor β, myosin light chain 2 and myosin binding protein C
- 2 proteins – YM1 and YM2 are mammalian chitinases, induced by IL13
  - associated with human asthmatics

Proteomic Analyses Complement Genomic and Functional Analyses

- Strength of proteomics is ability to evaluate multiple compartments in humans and animal models for translational investigations
  - Sputum, BAL, blood
- Good correlation of functional responses with protein expression
  - Mammalian chitinases, inflammatory proteins, secretory products
- Additional studies needed to assess utility to differentiate respiratory and dermal sensitizers
  - Multi-compartment analysis in chemical-induced asthma
    - Haenen *et al*. (2010) *J. Proteome Res* 9:5868-5876
      - Functional effects correlated with markers of neutrophilic inflammation and oxidative stress in lymph node, lung and BAL

Remember… *What You Find Depends On When You Look*

- Aerosol exposure of mice to Ova or saline for 1, 5, or 10 wks (ST, IT, LT groups)
- Functional, inflammatory, morphologic and gene expression changes measured
- Genes for cell division up-regulated during ST and IT
  - Down regulated during LT
- Genes linked to growth, differentiation, matrix metalloproteinases/collagens up-regulated in IT group
- Genes linked to mucous secretion progressively amplified



Di Valentin et al., (2009) Am J Physiol Lung Cell Mol Physiol 29: L185-L195

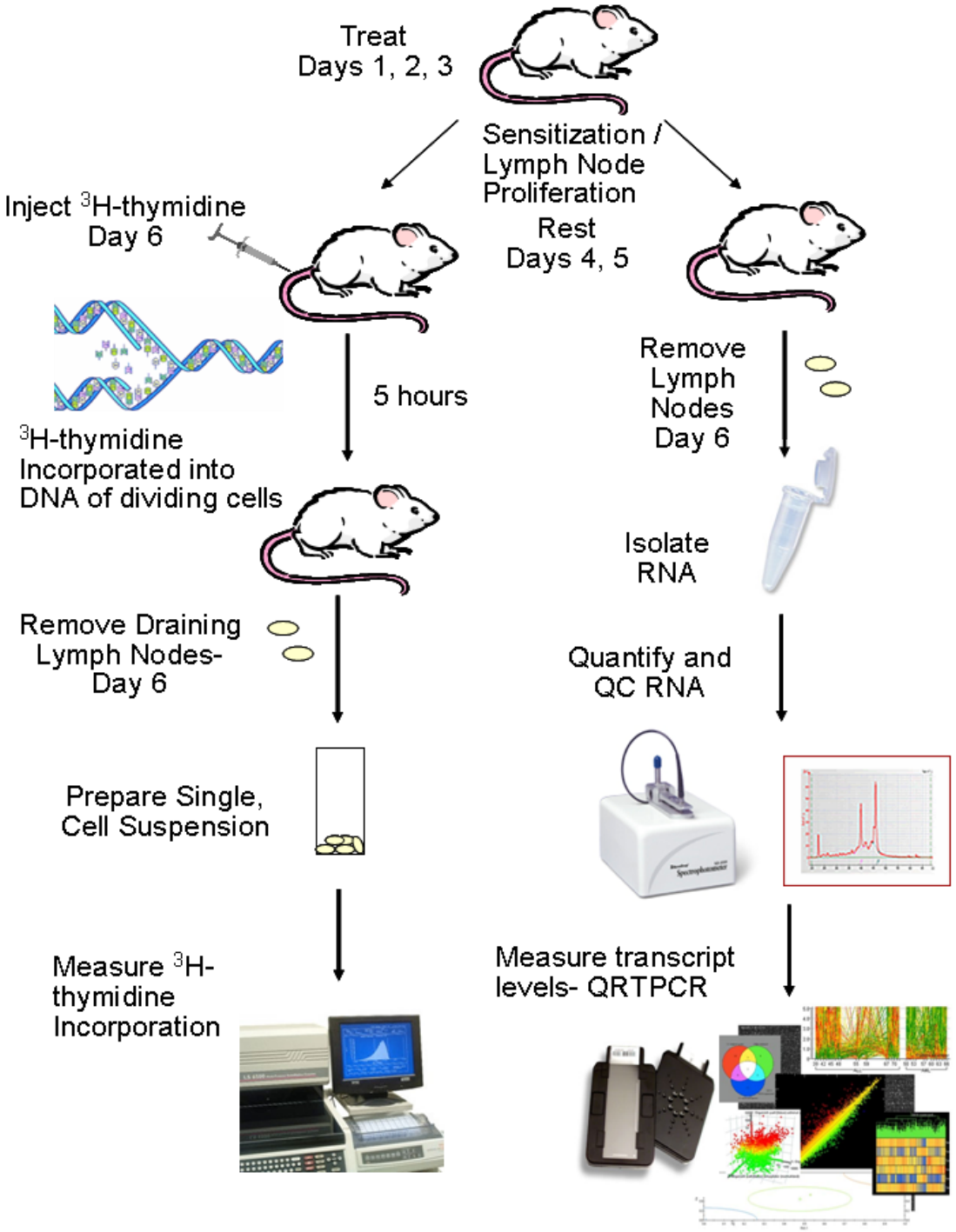
Can Genomics Enhance Identification of Respiratory Sensitizers?

Objective

- Expand on preliminary research on a toxicogenomic approach to enhance the LLNA- *Boverhof et al. 2009- Tox Sci 107(2), 427–439*
- Apply this approach to a more diverse array of chemicals to more fully assess the ability to:
  - Identify transcript markers of proliferation/potency
  - Distinguish non-sensitizing irritants from sensitizers
  - Differentiate dermal and respiratory sensitizers
- Test materials evaluated
  - Dermal sensitizers
    - DNCB (Dinitrochlorobenzene) and HCA (alpha-Hexylcinnamaldehyde)
  - Respiratory sensitizers
    - TMA (trimellitic anhydride) and OPA (ortho-phthalaldehyde**)**
  - Non sensitizing irritants
    - MS (methyl salicylate) and NA (nonanoic acid)

Approach

- Keep the toxicogenomic data anchored to the traditional LLNA design and endpoint
- Dose Response
  - 3 doses
- Endpoints:
  - ^{3}HTdR
  - gene expression

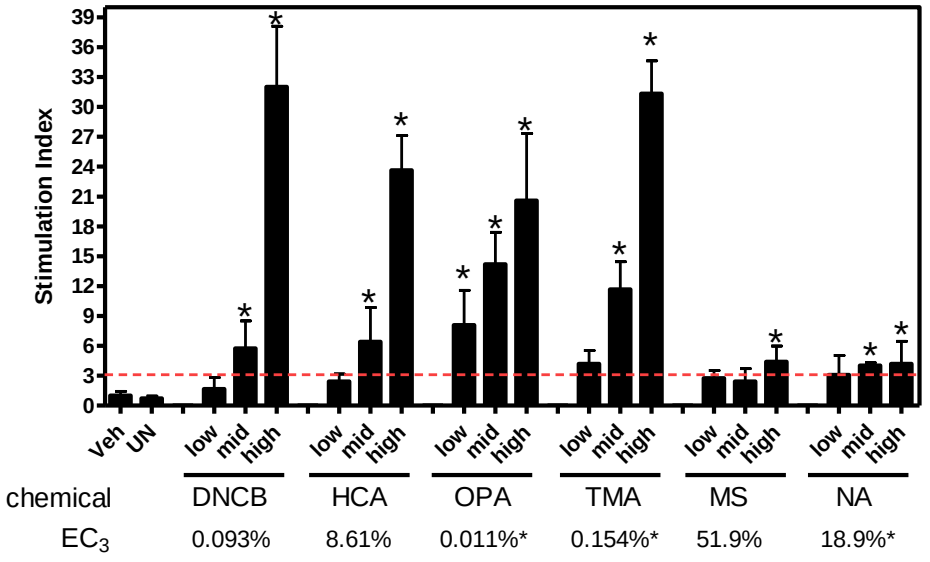


Boverhof et al., 2009- Tox Sci
Adenuga et al., 2012- Tox Sci

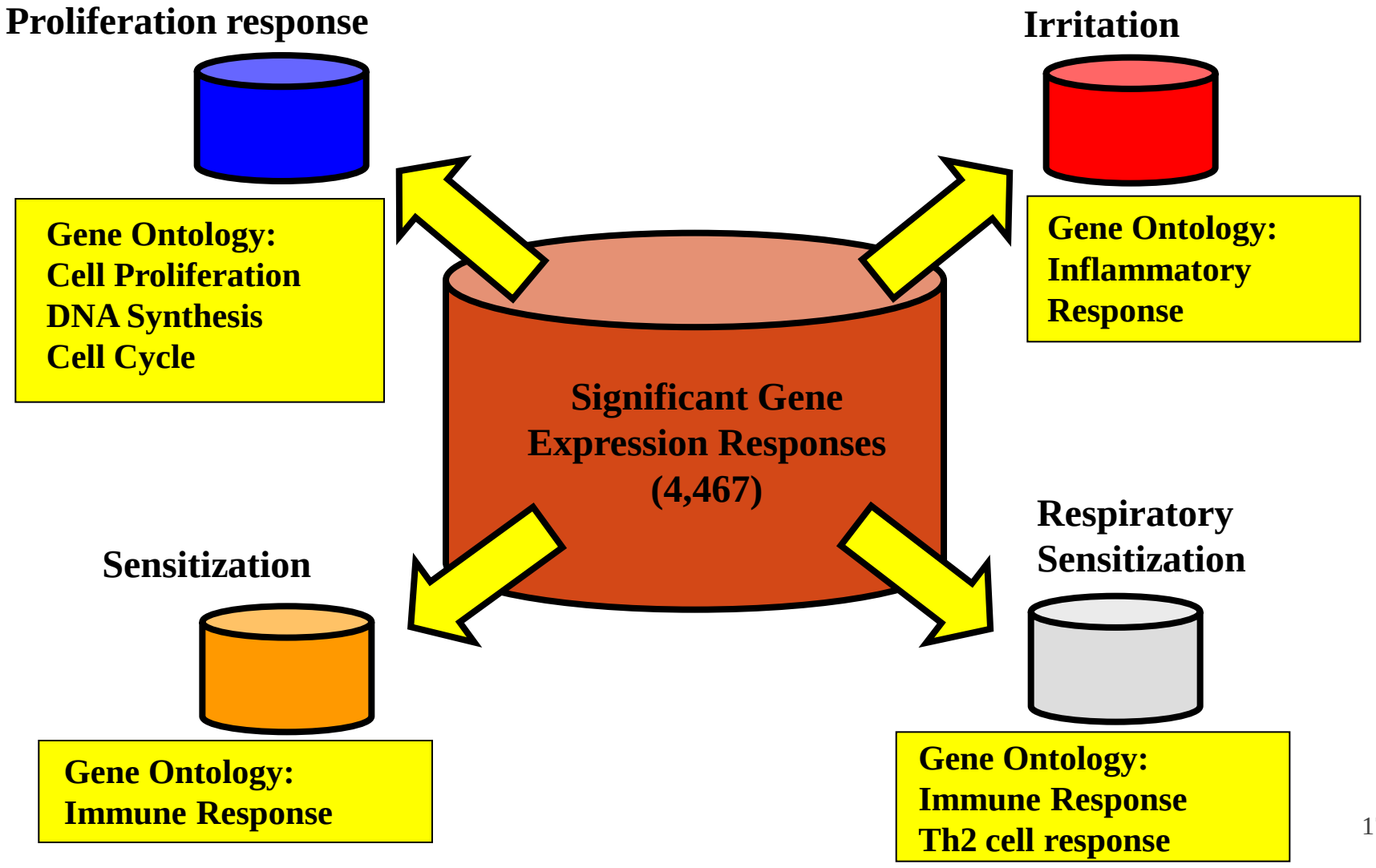
Dose Selection Is Important

- Sensitizers vary in potency
  - Doses for sensitizers were chosen to yield comparable LLNA responses across the chemicals.

Chemical	Dose		
	Low	Mid	High
DNCB	0.04%	0.20%	1.00%
HCA	7.50%	15%	45%
OPA	0.02%	0.04%	0.20%
TMA	0.20%	1%	5%
MS	20%	40%	80%
NA	20%	40%	80%

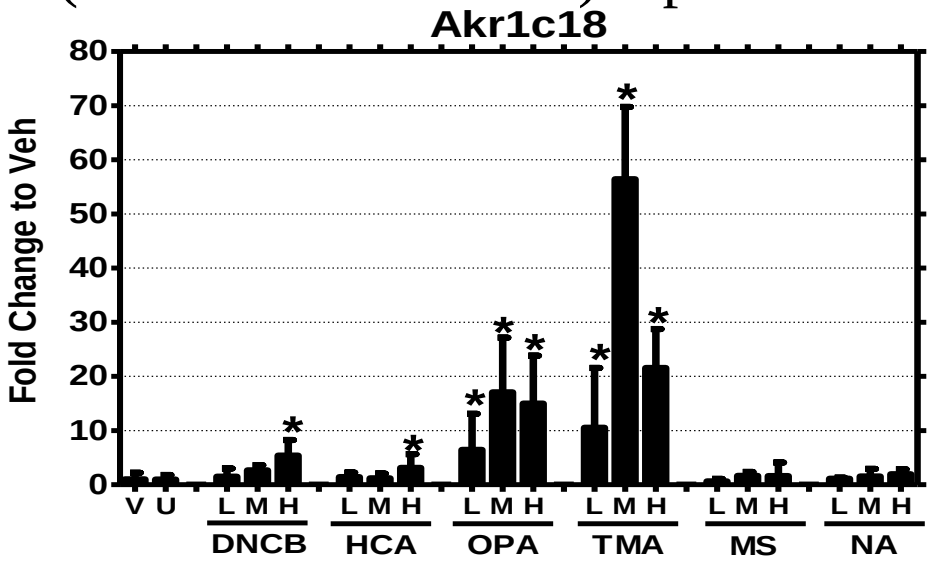


Data Mining/Stratification Identified Similarities With Elicitation Model



Respiratory Sensitizer-specific Transcripts Identified

- AKR1c18 (aldo-keto reductase) – promotes Th2 cell survival



- Galectin-7 – Cell-cell and cell-cell matrix interactions
- Mcpt1 and 8 – mast cell protease 1 and 8
- Frzb – Frizzled-related protein – cell differentiation
- Cd160 – NK cell and CD8 T lymphocyte marker
- IL4 – promotes development of Th2 bias

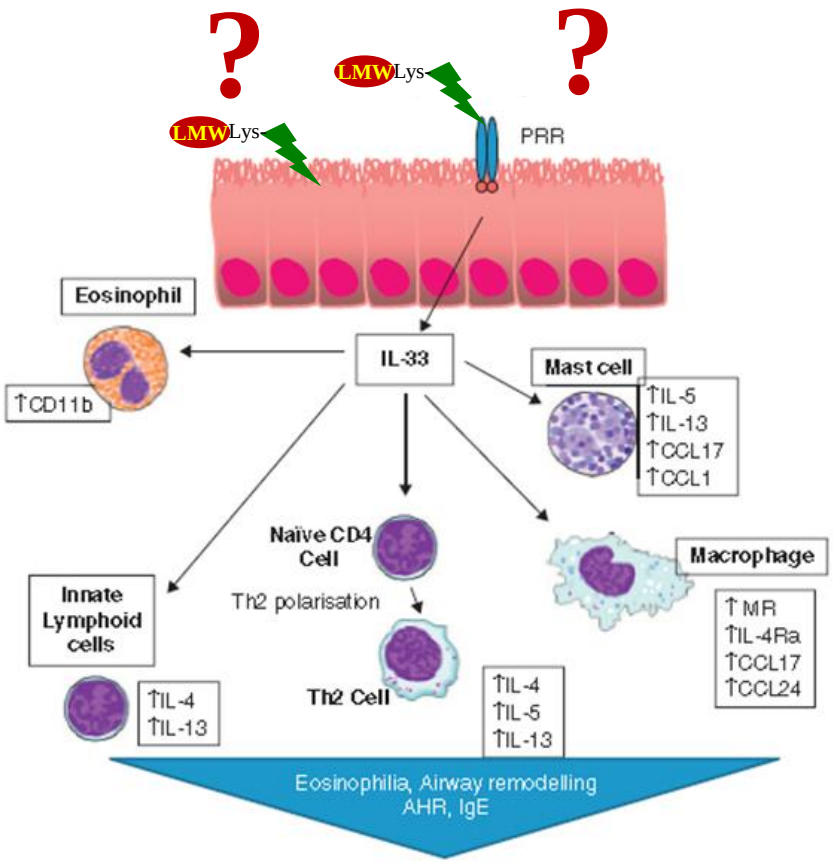
Genomics Show Promise to Differentiate Respiratory and Dermal Sensitizers

- Gene expression changes during sensitization (induction-phase) may enhance weight of evidence approaches to distinguish
  - Sensitizers from Irritants
  - Respiratory Sensitizers from Dermal Sensitizers
- Need to expand the LMW chemical data-set to confirm-extend gene expression signatures
- Expand analyses to upper/lower airway tissues to explore mucosal gene expression signatures

Question….

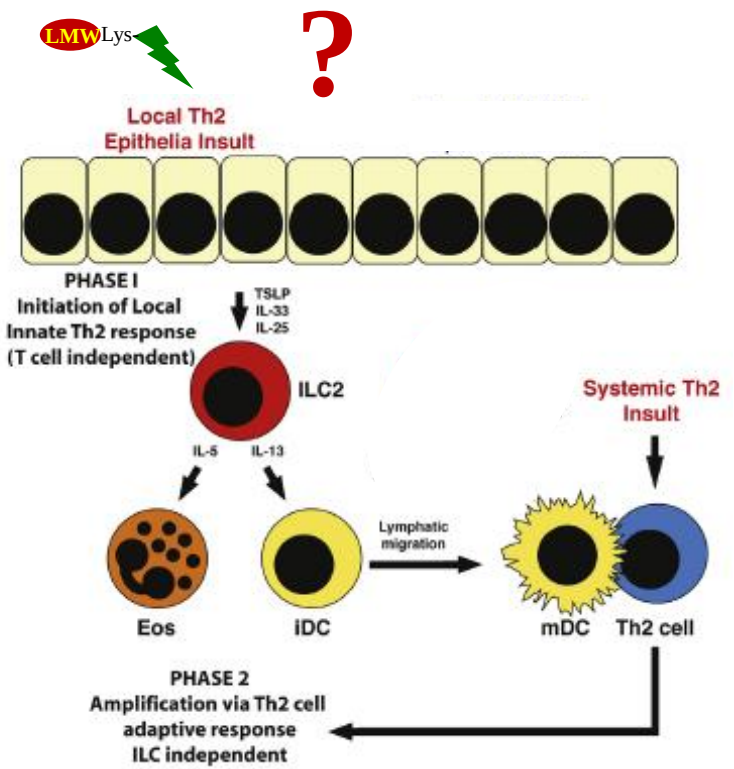
- **Why aren't all chemical sensitizers Respiratory Sensitizers?**

Initial Molecular Interactions and Airway Mucosal Responses May Drive Selective Th2-bias and Respiratory Sensitization



Adapted from Lloyd, CM (2010)
Current Opinions in Immunology 22:800-806

ILC2 Can Facilitate Allergic Sensitization



Adapted from Gold, MJ, et al (2014)
J. Allergy Clin Immunol 13:1142-1148

Closing Thoughts….

- A weight of evidence approach is currently required to differentiate Respiratory from Dermal sensitizers
  - Regulatory frameworks accept that dermal sensitizer assays will detect both dermal and respiratory sensitizers
- It is essential to develop and validate robust assay systems to distinguish Respiratory Sensitizers from Dermal Sensitizers and Irritants
- A science-based determination of sensitizer potency and thresholds of sensitization/elicitation is critical to address possible classification as SVHC under the "equivalent level of concern " route set out in Article 57(f) of REACH
- 'Omics' show great promise to identify key cellular and molecular events relevant to development of an adverse outcome pathway for respiratory sensitizers

Thank You

Questions?