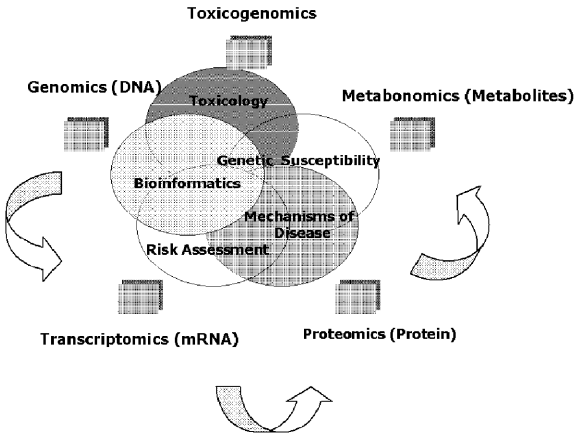
RJRT
Molecular Genetics Program

Objectives:

- Understand genetic changes in smoking–related diseases
- Understand potential genetic mechanisms of smoking-related diseases
- Understand genetically-based correlations of pre-clinical models of smoking-related disease with human diseases
- Evaluate the potential of PREPs to alter gene expression profiles associated with smoking related diseases

Interdisciplinary Aspects of Molecular Toxicological Analyses



Looking at expression at transcript level & protein

Interest in investigation metabolites

Toxicogenomics requires all of this

Genetic toxicological testing has broadened over the past decade (Zucco, 2004). The emergence of –omics technologies partly due to the Human Genome Project (which fostered the sequencing of the entire human genome) has expanded toxicological testing to include toxicogenomics.

Toxicogenomics is a combination of genomics and bioinformatics to characterize and identify mechanisms of toxicity induced by various chemicals including drugs.

Within this scientific discipline, genomics, transcriptomics, proteomics and most recently metabonomics have been applied to assess the toxicological responses of chemicals using *in vitro* and *in vivo* models and to assist with predictive toxicology, mechanistic toxicology, risk assessment and discerning mechanisms of disease (i.e. lung cancer).

Research Capabilities

- **Genomics**
  - Gene Expression Assays
    - Internal: QRT/PCR, PCR Array
    - Contracted: Membrane and Affymetrix Arrays
  - Gene Expression Data Analysis:
    - GeneSifter
    - Pathway Studio
- **Protein Analyses**
  - Multiplex protein analysis
  - Western Blot analysis
- **DNA Methylation**
  - Collaboration with Jay Goodman (Michigan State)
- **High Throughput Assays for Screening Oxidative Stress, Inflammation and Gene Promoter Activation**
  - Enzyme Linked Immuno Sandwich Assay (ELISA), Luciferase Reporter Cells

Future Molecular Endpoints

- Gene specific methylation profiles
- RNA regulation
  - Micro (mi)RNA
  - siRNA (RNA silencing)
- Single nucleotide polymorphisms (SNPs)
- Protein arrays
  - Surface markers (phenotypic changes)
- Inflammatory protein expression
  - Tissue correlation to serum markers
- Exon arrays

Program Objectives
Integrate Divisions of Product Integrity

- Preclinical Models of Disease
  - Model Development
    - *In Vitro* Lung and CVD models
    - *In Vivo* Lung, COPD and CVD models
- External Research
  - Duke University
    - David Harpole
    - Wolgang Leidtke and Sid Simon (Postdoctoral Research Program)
  - Gene Logic, Inc.
  - Michigan State
    - Jay Goodman
  - BAT Projects (Contracts / Potential Collaborations)
- Human Studies (Discussions in progress)

Research for Oral Tobacco Products and Human Correlations

- Investigate gene expression profiles and inflammatory markers
  - Buccal cells exposed to whole smoke and OTP extracts
  - Lymphocyte / Peripheral blood mononuclear cell analysis from smokers and non-smokers
  - Assess inflammatory markers in serum (rodent and human)

RJRT - BAT

Contracted Services

- TOX 187 - 8 Cigarette study (RJRT contract services for BAT)
- Eclipse testing (BAT contract services for RJRT)

Potential Collaborations

(pending further discussion and approval)

- MicroRNA analysis of rodent tissue, human lung cells and human lung tissue
- Molecular techniques for *in vitro* models of CVD and lung disease

TOX 187
BAT contracted 8 Cigarette study

1) Lung and liver samples from Sham and high dose BAT reference cigarette collected
2) BAT – Affymetrix gene expression profiling – results due 1Q08
3) RJRT – RT-PCR analysis of selected genes – 50 % complete in 2007
4) Jay Goodman – gene methylation profiling of lung - complete

TOX 187 - 8 Cigarette Study
(RJRT contract services for BAT)

- Lung and liver samples collected from sham and high dose BAT reference cigarettes
- BAT – Affymetrix gene expression profiling
  - Complete
- RJRT – PCR Array analysis
  - Initial analysis complete
  - Affymetrix follow-up analysis – suspended by BAT/RJRT due to low statistical power of the Affymetrix results
- Jay Goodman – gene methylation profiling of lung
  - Complete

TOX 187
BAT contracted 8 Cigarette study

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Eclipse Testing

(BAT contract services for RJRT)

Objective: Evaluate and compare effects of 2R4F and Eclipse on gene expression profiles and cytokine release in human H292 cells

- Cytokine analysis
  - Complete
- Gene expression profiles
  - Complete but variable

Next steps:

- Issue BAT gene expression and cytokine data as RJRT RDM (internal review)
- Evaluate feasibility of publishing data (3Q08)

Gene Methylation
Michigan State - Jay Goodman
RJRT Collaborative Studies

Objective: Examine gene methylation patterns in animal models to evaluate use as a biomarker of effect

- SENCAR mouse skin – CSC promotion
- SENCAR mouse lung – whole smoke inhalation

SENCAR Mouse Skin – CSC Promotion

(Michigan State – Jay Goodman)

- Tumor tissue from CSC-promoted animals globally hypomethylated compared to non-tumor tissue, GC specific methylation higher in tumors (published, Tox. Sci. 2003).
- Increased methlyation of HOX A5 promoter region correlates with decreased expression of the gene during CSC tumor promotion (published, Mol. Carc. 2004).
- Altered methylation in gene-specific and GC-rich regions is progressive and non-random during CSC promotion (published, Tox. Sci. 2006).

SENCAR Mouse Lung – Whole Smoke Inhalation

(Michigan State – Jay Goodman)

- Cigarette smoke causes formation of regions of altered DNA methylation in the lung of Sencar mice (SOT, 2008).

RJRT Studies

- Develop RT/PCR system in NHBE cells (published Tox. Meth. 1999).
- Conduct gene expression profiling in NHBE cells (published Tox. Sci. 2001).
- Evaluate effects of chemical treatment (cycloheximide, MNNG) on gene expression profiles in NHBE cells (published Tox. Sci. 2001).
- Evaluate effect of B(a)P and metabolites on c-myc expression in NHBE cells (published Mol. Carc. 2004).
- Matrix-degrading and pro-inflammatory changes in human vascular endothelial cells exposed to cigarette smoke condensate. (published Cardiovascular Tox. 2003).
- Evaluate and compare effects of 1R4F and Eclipse CSC on gene expression in NHBE cells (published Tox. Sci. 2005).
- Analysis of cytokine responses to CSC in human endothelial and monocytic cells. (published Toxicology, 2005).

RJRT Studies (continued)

- Evaluate effects of 1R4F and 2R4F whole smoke on gene expression in NHBE cells (submitted Tox. Sci. 2007).
- Evaluate and compare effects of 2R4F and Eclipse whole smoke on gene expression in NHBE cells (2008 AACR meeting).
- Evaluate the time course of expression of genes involved in specific pathways in normal human bronchial epithelial (NHBE) cell genes following exposure to cigarette smoke (Accepted - Experimental Lung Research, 2008).
- Evaluate human inflammatory cytokines and receptors responses to CSC in normal human bronchial epithelial (NHBE) cells. (Accepted - Journal of Cytokine & Interferon Research, 2008).
- MMP-1 polymorphic expression in aortic endothelial cells: possible role in lesion development in smokers and nonsmokers. (published Cardiovascular Tox. 2004).

2008 Program Deliverables

- Identify differences in gene expression in human lung cancer (adenocarcinoma and squamous cell carcinoma) and adjacent pathologically normal tissue.
- Identify differences in gene expression between smoker and non-smoker normal lung tissue and smoker COPD tissue.
- Identify changes in gene expression following smoke exposure using *in vitro* and *in vivo* models of lung cancer, COPD and CVD.