Radiation Effects on Metabolic Gene Expression

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About Calvin

• From Eden Prairie MN
• Bethel University, St. Paul MN
  – Chemistry and Biochemistry
• Enjoy the outdoors
  – Camping
  – Skiing
• Electronics!
• Mission: "To optimize human health and productivity for space exploration"

• Human Adaptation and Countermeasures Division
  – Understand the normal human response to space flight
  – Develop countermeasures to protect crew health
Radiation

• Sources:
  – Galactic cosmic rays
  – Solar particle events

• Concerns:
  – DNA damage
  – Increase in oxidative products

• Health outcomes:
  – Cataracts
  – Cancer
  – Central nervous system damage
  – Radiation sickness
  – Unknowns
Pharmacology

• Medications are vital to human spaceflight
• Spaceflight induces changes in human physiology
• Yet drug metabolism is assumed to be the same as on earth
  – Is this assumption valid?
Research

• Challenge of spaceflight: **Radiation**
• Application: **Drug Metabolism** in the liver

• In General, activity of liver metabolic enzymes determines the concentration of circulating drugs
  – Decreased liver function = drug overdose
  – Elevated liver function = ineffective treatment
Goal

• Identify how radiation exposure affects transcriptional gene expression in the liver
  – Focus on genes associated with:
    • Drug Metabolism
    • DNA Repair
• Foundation for implementing countermeasures
Method Overview

**RNA Extraction & Purification**

Liver Tissue → RNA

**Reverse Transcription**

RNA → cDNA

**RT-qPCR**

cDNA → Gene Expression

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Methods: Ground Model

• Male C57 mice exposed to $^{137}$Cs in 4 Groups:
  – Control
  – Low dose (50 mGy)
  – High dose (6 Gy)
  – Both radiation doses (low then high) separated by 4 hours
• Each group contained 6 mice
• 4 sets sacrificed at 4 hours, 24 hours, 7 days and 13 days after their last radiation exposure
  – I worked with the 7 day set
• Livers flash frozen in liquid nitrogen
Methods: RNA Extraction

• Agilent Absolutely RNA Miniprep Kit
  – Tissue homogenized in a lysis buffer
  – Prefiltered sample in a spin cup
  – RNA-binding spin cup
    • Series of washes remove DNA and proteins
  – Highly pure RNA is eluted into a microcentrifuge tube using an elution buffer
Methods: RNA Quality Testing

• Agilent 2100 Bioanalyzer with Agilent RNA 6000 Nano kit
  – Microfluidic chip
• Check total RNA integrity and concentration
• RNA integrity number (RIN)
  – Samples must have RIN > 8
Methods: Reverse Transcription

- SABiosciences RT2 First Strand Kit
  - cDNA prepared from RIN > 8 RNA samples
- PCR requires DNA
  - In vitro transcription by mRNA and enzymes:
    - Reverse transcriptase and DNA polymerase
Methods: RT-qPCR

• Real-time polymerase chain reaction
• Measure gene expression
• SABiosciences RT2 Profiler Arrays
  – DNA Repair and Drug Metabolism
  – Test many genes simultaneously
  – 96-well plates
    • 84 pathway focused genes
    • Controls for testing inter-well and intra-plate consistency
  – SYBR Green Detection
Calculations

• Analyzed $C_t$ data from all data sets
  – 72 mice $\times$ 89 genes per plate $\times$ 2 plates
    $= 12,816$ genes total
• Set RT-qPCR baseline at 600
• Determined gene expression by $\Delta\Delta C_T$ method
• Normalized data to housekeeping genes
  – Drug Metabolism: Adh1, Blvrb, Gstm4, Gstm5, Marcks, Snn
  – DNA Repair: Polb, Rad21, Rpa3, Slk, Tdg, Xrcc4

$$2^{-\Delta C_t(\text{GOI})} \frac{\text{expt}}{\text{control}} = 2^{-\Delta C_t(\text{HKG})} \frac{\text{expt}}{\text{control}} = 2^{-\Delta C_t} \frac{\text{expt}}{\text{control}} = 2^{-\Delta \Delta C_t}$$
Drug Metabolism Results

Gene Expression

Mt2: Metallothionein

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Drug Metabolism Results

Gene Expression at 24 Hours

- Fold Regulation
- Low (50 mGy)
- High (6 Gy)
- Both

Genes: gsh2b1a, Adh4, Adh5, Gsr, Mthf, Nap1, Gmt1, Nat2, Gtm1, Gst17a1, Cyp1a2, Cyp2c9, Cyp4b1, Fad, Bhp1, Pon1, Gpx4, Gstm1, M2, Pkl, Pkn3, Ahr, Smarca1, Hsp90ab1, Actb

Categories: P-Glycoprotein, Oxidoreductases, Transferases, Cytochrome P450, Hydrolases, Metallothioneins, Kinases, Other
Drug Metabolism Results

Gene Expression at 7 Days

- **Gene**
  - Abcb4
  - Blvra
  - Cyp17a1
  - Cyp2c29
  - Cyb5r3
  - Ephx1
  - Gusb
  - Pkm2

- **Fold Regulation**
  - Low (50 mGy)
  - High (6 Gy)
  - Both

- **Cytochrome P450**
- **Hydrolases**
- **Kinases**

**Gene**
- **P-Glycoprotein**
- **Oxidoreductases**
DNA Repair Results

Gene Expression at 4 Hours

Fold Regulation

-4 -3.5 -3 -2.5 -2 -1.5 -1 -0.5 0 0.5 1

Apex1  Ung  Ercc3  Ercc8  Lig4  Xrcc2  Xrcc6bp1  Atr

Low (50 mGy)  High (6 Gy)  Both

Base Excision Repair  Nucleotide Excision Repair  Double-Strand Break Repair  Other

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DNA Repair Results

Gene Expression at 24 Hours

Nucleotide Excision Repair

Base Excision Repair

Double-Strand Break Repair

Mismatch Repair

Other

Gene

Lig2, Neln, Parp2, Parp3, Ung, Xrcc1, Ddb1, Ercc1, Ercc2, Ercc8, Rad23a, Rpa1, Xap2, Fen1, Lig4, Xrcc3, Mlh1, Mlh3, Msh3, Mgmt, Xrcobp1, Actb

Fold Regulation

High (6 Gy)

Both

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Gene Expression at 7 Days

- **Mms19**
  - Fold Regulation:
    - High (6 Gy): 2.5
    - Both: 1.5

Nucleotide Excision Repair

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Drug Metabolism Gene Relationships

- Down-regulation
- Up-regulation
- Regulation Direction Unknown
- Coexpression
- Chemical Modification
- Physical Interaction
- Predicted Protein Interaction
- Predicted TFactor Regulation
- Other

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DNA Repair Gene Relationships

- Down-regulation
- Up-regulation
- Regulation Direction Unknown
- Coexpression
- Chemical Modification
- Physical Interaction
- Predicted Protein Interaction
- Predicted TFactor Regulation
- Other

Diagram showing gene relationships with various interactions:

- XAB2
- RAD23A
- APEX1
- MMS19
- XRCC3
- LIG4
- LIG3
- ERCC8
- DDB1
- ERCC1
- MLH1
- MLH3
- NEIL1
- PARP2
- RPM1
- UNG
- FEN1
- ATR
Conclusions

• The expressions of 65 genes have been found to be affected by radiation exposure in mice
  – Effects vary with time and dose

• Radiation exposure effects metabolism of drugs with lipid or steroid hormone-like structures
Methods: Flight Samples

- 15 mice on STS-135!!!
  - 15 controls (Calcein)
  - 15 Baseline (2 weeks younger, no Calcein)
- Processed 6 control and 6 baseline samples
- Legal issues with flight samples....tbd
- Animal Enclosure Module (AEM)
Future Research

• Complete 13 Day and STS-135 data sets

• Evaluate altered genes at protein level

• Correlate findings with drugs used in spaceflight
  – Inform countermeasures
Sources

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Thank You!