Global transcriptomic profiling of microcystin-LR or -RR treated hepatocytes (HepaRG)

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Microcystin congeners

• 100s of congeners
• Differentiated by structure
• Occur in mixtures
• MC-LR best characterized
• MC-RR commonly found w/ -LR
Prototypical Toxicity Pathway

- **Ingestion**
  - Oral Ingestion
  - Absorption through GI -> Liver

- **Liver**
  - Phosphatase Inhibition
  - Hyperphosphorylation

- **Systemic**
  - Cytoskeletal rearrangement
  - Cell death
  - Hepatic bleeding

- **MC congeners poorly characterized**
  - No mechanistic data
  - Potential for unidentified mechanisms

- **MC-LR > -RR toxicity**
  - MC-LR ≈ -RR in PP inhibition
  - Toxicokinetics
  - Molecular targets?
Molecular characterization of MC targets

Gene transcription
- Underlies cellular processes
- Likely to be impacted via phosphatase inhibition
- Generation of hypothesis

MC-LR
1000, 100, 10 μg/L

MC-RR
Design and Overview

| Group | Total | Up-regulated | Down-regulated | 1 | 2 | 3 | N total |
|-------|-------|--------------|----------------|---|---|---|---------|
| LR10  | 339   | 230          | 109            | 7 | 6 | 6 | 19 |
| LR100 | 171   | 116          | 55             | 10 | 6 | 6 | 22 |
| LR1000| 2098  | 1740         | 358            | 6 | 6 | 6 | 18 |
| RR10  | 12    | 11           | 1              | 7 | 6 | 0 | 13 |
| RR100 | 1255  | 1130         | 125            | 10 | 6 | 6 | 22 |
| RR1000| 1279  | 1138         | 141            | 6 | 6 | 6 | 18 |
| Solvent| 18    | 12           | 6              | 6 | 6 | 6 | 36 |
## Cytotoxicity

| Treatment | XTT        |
|-----------|------------|
| LR10      | 0.0592     |
| LR100     | 7.24e-05   |
| LR1000    | 9.48e-12   |
| RR10      | 0.3178     |
| RR100     | 0.7634     |
| RR1000    | 0.4227     |
Consistency of DEGs and enrichment

| LR1000 | LR100 |
|--------|-------|
| FOS    | 15.5  |
| ATF3   | 8.22  |
| FOSB   | 6.31  |
| IER2   | 4.08  |
| RND1   | 3.94  |
| GDNF   | 3.69  |
| NFKBIZ | 3.08  |
| GADD34 | 3.00  |
| JUN    | 2.68  |
| SLC25A25 | 2.45 |

- LR1000:LR100 (59% overlap)
  - bZIP TFs
  - FOS family of proteins
  - TNF-α signaling pathway

- LR1000:RR1000 (25% overlap)
  - Aldehyde Dehydrogenase family
  - 7/8 members in common

- RR1000:RR100 (20% overlap)
  - Complement
  - Extracellular exosome
  - acetylation
Endoplasmic Reticulum Stress Response

Adaptive response
- IREα1
- XBP1
- HSP9C
- HSPA5
- PERK
- eIF2α
- Paused translation

Endoplasmic Reticulum C,F
- Increased protein folding capacity

Apoptosis
- PERK
- CHOP
- ATF4
- ATF3
- Gadd34
- eIF2α
- Translation

MC-RR
- MC-RR
- ROS/LPO

ROS/LPO
- MC-RR
- MC-LR

ER Stress
- PUMA/NOXA/C/BIM
- DR5A
Other common identified targets/processes

• Lipotoxicity
  • ALDH family up-regulated in MC-LR & - RR
  • ROS

• Extracellular Exosomes
  • Highly enriched in MC-RR
  • Less so in MC-LR
  • Off-loading misfolded proteins?
  • Extracellular signaling - sensitization
MC-LR specific

• AP-1 constituents
  • Among the most consistently and highly expressed
  • FOS, JUN, ATF
  • TNF-α/JNK signaling

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\text{TNF-\(\alpha\)} \rightarrow \text{JNK} \rightarrow \text{AP-1: FOS/JUN} \rightarrow \text{p53} \rightarrow \text{PUMA/NOXA/BIM} \rightarrow \text{Apoptosis}
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\[
\text{NFkBIZ} \rightarrow \text{NF-\(\kappa\)B} \rightarrow \text{Inflammatory Response}
\]

• Protein Phosphatases – PP1 and 2
MC-RR Specific Response

- Enrichment of complement genes
  - Immunity and defence
  - Liver damage → Apoptosis

- Diversity of protein phosphatases
  - Little overlap with MC-LR
Conclusions

• Consistent response
  • w/in study and across
• Oxidative Stress is a key driver of MC toxicity
• ER-stress is important
• MC-LR and –RR differ
  • Toxicity
  • PP targets
• Toxicity confounded with congeners
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