Integrating Literature Analysis into the NTP Research Pipeline

Windy Boyd
NTP Board of Scientific Counselors Meeting
December 12, 2018
DNTP Translational Toxicology Pipeline Plan

Define Hypotheses & Design a Testing Strategy

- Data Mining
- QSAR Profiling
- Bioactivity Screening
- In vitro Studies
- Longer-term in vivo Tests
- Short-term in vivo Tests
- Knowledge Integration

Communicate

Evidence Mapping

Inform Public Health Decisions

Systematic Review

Literature Analysis
Evidence Mapping
- Inform Research
  - Data pockets
  - Data gaps

Literature Analysis
- Define Hypotheses & Design a Testing Strategy
  - In vitro Studies
  - Short-term in vivo Tests
  - Longer-term in vivo Tests
  - Knowledge Integration

Data Mining
- QSAR Profiling
- Bioactivity Screening

Inform Public Health Decisions
Parkinson's disease (PD) due to progressive neurodegeneration

- Aggregation of α-synuclein in Lewy bodies
- Loss of dopaminergic neurons in substantia nigra
- Signs include tremor, rigidity, and shuffling gait

Highly prevalent but etiology of most PD cases unknown

- Genetics only account for ~10% of cases
Environmental factors

- Exposures to pesticides linked to Parkinson’s in epidemiological studies
- Need for better understanding of which environmental factors may be contributing and how they act
- Neurodegeneration is not included in routine toxicological testing strategies
- Lack of methods to rapidly identify environmental exposures
Project team

- Combined scientific expertise in neurotoxicology, *in vitro* screening, toxicoinformatics, and literature analysis

Goals

- Identify previously evaluated chemicals, genes and pathways, and model systems
- Develop a battery of *in vitro* and alternate model organism assays to screen chemicals for potential effects
NTP Parkinson’s Disease Project

Strategy to identify potential chemical contributors

- Expert knowledge
- Published literature
- Tox21 HTS data
- Chemical and assay selection
- Toxicogenomic databases
• Questions: Which chemicals, genetic targets, and models have been reported in the scientific literature?

• PubMed search identified >90,000 records with mention of Parkinson’s disease

• Screened studies for environmental chemical exposure and categorized by study characteristics
Exposures Associated with Parkinson’s Disease

Automated Tagging of All Environmental Exposures

- Metals (1734)
- Smoking (1163)
- Alcohol (778)
- Occupational (442)
- Air Pollution (235)
- Miscellaneous (172)
- PAHs (35)
- Stress (4339)
- Drugs of Abuse (2654)
- Nutrition (2490)
- Pesticides (2576)
Manual categorization of 1,840 studies revealed similar trend as automated tagging and allows researchers to explore published literature.
## Parkinson’s Disease Evidence Map

### Most-reported Environmental Chemicals

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Effect</th>
<th>Human</th>
<th>In vivo</th>
<th>In vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manganese</td>
<td>Exposure</td>
<td>115</td>
<td>106</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>Positive control</td>
<td>6</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Paraquat</td>
<td>Exposure</td>
<td>22</td>
<td>135</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Positive control</td>
<td>37</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Rotenone</td>
<td>Exposure</td>
<td>10</td>
<td>137</td>
<td>198</td>
</tr>
<tr>
<td></td>
<td>Positive control</td>
<td></td>
<td>204</td>
<td>274</td>
</tr>
<tr>
<td>Nicotine</td>
<td>Exposure</td>
<td>21</td>
<td>36</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>16</td>
<td>61</td>
<td>18</td>
</tr>
</tbody>
</table>
### Environmental Chemicals in >10 Studies

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Human</th>
<th>In vivo</th>
<th>In vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maneb</td>
<td>8</td>
<td>43</td>
<td>22</td>
</tr>
<tr>
<td>Aluminum</td>
<td>17</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Iron</td>
<td>17</td>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>10</td>
<td>6</td>
<td>23</td>
</tr>
<tr>
<td>Mercury</td>
<td>19</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Copper</td>
<td>10</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Lead</td>
<td>20</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Cadmium</td>
<td>5</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>PCBs</td>
<td>9</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Zinc</td>
<td>8</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Mancozeb</td>
<td>1</td>
<td>7</td>
<td>4</td>
</tr>
</tbody>
</table>

- Very few chemicals with multiple reports
- All metals and/or pesticides except PCBs
- Many chemicals with single study (not shown)
Candidate Chemical Library

• Predicted actives
  – Positive controls
    • MPTP, rotenone, paraquat
  – Metals and metal compounds
    • Manganese tricarbonyl (MMT), maneb, methyl mercury, ziram
  – Organochlorines
    • DDT, heptachlor, dieldrin, lindane, endosulfan, TCE, hexachlorobenzene
  – Organophosphates
    • Chlorpyrifos, diazinon
  – Other pesticides
    • Permethrin, benomyl, tributyltin methacrylate, quintozene

• Unknowns
  – Triphenyl phosphate
  – Isopropylated phenyl phosphate
  – Captan
  – Glyphosate
  – Pyridaben
  – Acetaminophen

• Predicted Negatives
  – Saccharin sodium
  – L-ascorbic acid
  – D-glucitol
  – Acetyl salicylic acid
Informing Assay Selection for Targeted Testing

Motor deficits

Related changes in gene expression

Neuroinflammation

Mamta Behl
Tox Branch
### Parkinson’s Disease Evidence Map

**in vitro** Effects of Paraquat Exposure

*Some studies may have characterized multiple health effects or species and therefore may be represented multiple times. Row and column grand totals represent counts of distinct references.*

<table>
<thead>
<tr>
<th>Effect</th>
<th>Human</th>
<th>Rat</th>
<th>Mouse</th>
<th>Rat x Mouse</th>
<th>Bovine</th>
<th>Grand Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>DA (TH+) neurons</td>
<td>2</td>
<td>8</td>
<td>9</td>
<td></td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>Dopamine (DA and metabolite levels, DAT and receptor expression, TH immunoreactivity)</td>
<td>4</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>alpha synuclein, Tau phosphorylation, tubulin</td>
<td>11</td>
<td>4</td>
<td>2</td>
<td></td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>Proteasome (Parkin, proteasomal activity)</td>
<td>10</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>Mitochondrial effects</td>
<td>22</td>
<td>13</td>
<td>2</td>
<td></td>
<td></td>
<td>37</td>
</tr>
<tr>
<td>Other (general expression changes, etc.)</td>
<td>40</td>
<td>28</td>
<td>13</td>
<td></td>
<td></td>
<td>78</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>40</td>
<td>39</td>
<td>13</td>
<td>2</td>
<td></td>
<td>89</td>
</tr>
<tr>
<td>Cell viability (LDH levels, apoptosis, total cell number)</td>
<td>65</td>
<td>60</td>
<td>19</td>
<td>2</td>
<td>1</td>
<td>137</td>
</tr>
<tr>
<td>Grand Total</td>
<td>81</td>
<td>74</td>
<td>30</td>
<td>2</td>
<td>1</td>
<td>178</td>
</tr>
</tbody>
</table>
Reported *in vitro* Models

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Category</th>
<th>Cell, tumor, subfraction type</th>
<th>Tissue origin</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH-SY5Y</td>
<td>tumor</td>
<td>neuroblastoma</td>
<td>brain, bone marrow metastasis</td>
<td>49</td>
</tr>
<tr>
<td>SK-N-SH</td>
<td>tumor</td>
<td>neuroblastoma</td>
<td>nerve, bone marrow metastasis</td>
<td>7</td>
</tr>
<tr>
<td>primary mesencephalic cultures</td>
<td>primary</td>
<td>neurons</td>
<td>mesencephalon</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>neurons, glia</td>
<td>mesencephalon</td>
<td>2</td>
</tr>
<tr>
<td>PC12</td>
<td>tumor</td>
<td>pheochromocytoma</td>
<td>adrenal gland</td>
<td>20</td>
</tr>
<tr>
<td>N27</td>
<td>transformed</td>
<td>neurons</td>
<td>mesencephalon</td>
<td>17</td>
</tr>
<tr>
<td>primary cerebral cortex cultures</td>
<td>primary</td>
<td>glia</td>
<td>cerebral cortex</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>microglia</td>
<td>cerebral cortex</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>neurons</td>
<td>cerebral cortex</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>neurons, glia</td>
<td>cerebral cortex</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>oligodendrocyte progenitors</td>
<td>cerebral cortex</td>
<td>1</td>
</tr>
<tr>
<td>primary cerebellar cultures</td>
<td>primary</td>
<td>granule neurons</td>
<td>cerebellum</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>neurons</td>
<td>cerebellum</td>
<td>3</td>
</tr>
<tr>
<td>BV-2</td>
<td>transformed</td>
<td>microglia</td>
<td>brain</td>
<td>5</td>
</tr>
<tr>
<td>primary astrocytes</td>
<td>primary</td>
<td>astrocytes</td>
<td>brain</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>cerebral cortex</td>
<td>1</td>
</tr>
<tr>
<td>brain cultures</td>
<td>ex vivo</td>
<td>mixed</td>
<td>brain</td>
<td>1</td>
</tr>
</tbody>
</table>

✓ majority of studies conducted in human and rat tumorigenic cell lines with fewer more relevant, complex models
Toxicoinformatic Analysis

- Selected genes associated with Parkinson’s
  - Illumina’s NextBio datamining software
  - Comparative Toxicogenomics Database (CTD)

- Grouped 233 genes into 15 disease-relevant pathways

- Linked genes to studies included in literature analysis

- Gene expression reported in 47% of relevant studies
  - 57% of 233 genes evaluated in those studies
Identifying Chemical-Gene Combinations

Chemical Classes
- Pesticides
- Nicotine
- Metals
- Other

Biological Pathways
- Apoptosis
- Adenosine Receptor
- Axon guidance/synaptic
- Dopamine
- Dopamine Receptor
- Immune System
- α-synuclein/Lewy body
- Neuronal survival/activity
- Other
- Other receptors
- Mitochondria/ox phos
- Transcription factor
- Transporter
- Ubiquitin

Nisha Sipes
BSB
NTP Parkinson’s Disease Project

Strategy to identify potential chemical contributors

- Expert knowledge
- Published literature
- Tox21 HTS data
- Chemical and assay selection
- Toxicogenomic databases
DNTP Translational Toxicology Pipeline Plan

Define Hypotheses & Design a Testing Strategy

- Bioactivity Screening
- QSAR Profiling
- Data Mining

ADME/Chemistry

In vitro Studies

Short-term in vivo Tests

Longer-term in vivo Tests

Knowledge Integration

Communicate

Inform Public Health Decisions

Evidence Map

Systematic Review

Informed

Next

Future?
Acknowledgements

NTP/NIEHS

• Nisha Sipes
• Mamta Behl
• Andy Rooney
• Vickie Walker
• Scott Auerbach

OHAT

• Brandy Beverly
• Kembra Howdeshell
• Kyla Taylor

External

• Kris Thayer, former project lead, US EPA
• Ana Antonic, University of Melbourne
• Courtney Skuce, Robyn Blain, Pamela Hartman, Kelly Shipkowski, Sophie Hearn, ICF
• Austin Wray and Aaron Niman, US EPA

Peer Review

• Chris McPherson, Paraquat Scoping Report
• Nisha Sipes, Paraquat Scoping Report
Thank you

Questions?

…on behalf of OHAT