Follow the nose: using whole-skull IHC to map olfactory involvement in Parkinson disease

Fanyi Meng1, Li Dong2, Louise Pelletier2, Julianna J. Tomlinson3, Earl Brown4, Michael G. Schlossmacher1,3

1Faculty of Medicine, University of Ottawa, Ontario, Canada
2Department of Pathology and Laboratory Medicine, University of Ottawa, Ontario, Canada
3Division of Neuroscience, Ottawa Hospital Research Institute, Ontario, Canada
4Biochemistry, Microbiology & Immunology, Faculty of Medicine, University of Ottawa, Ontario, Canada

INTRODUCTION

Parkinson disease (PD) is a neurodegenerative disease characterized by the dysregulation and deposition of α-synuclein in the central nervous system, producing Lewy bodies in a stereotypic and progressive pattern. The average age of onset is 60 years, though it is known that olfactory impairment and gastrointestinal dysfunction may precede clinical parkinsonism by decades. In 2003, Braak and colleagues postulated the “dual-hit” hypothesis, whereby the PD process begins after an environmental toxin enters the CNS through either a nasal or a gastric route (or both), then spreads trans-synaptically into vulnerable brain regions. This hypothesis is supported by a Lewy body deposition pattern that correlates with clinical disease progression. Though research on the gastric pathway in mouse models has been productive, studies on the nasal pathway has been sparse due the difficulties in visualizing the nasal sinuses and cranial nerves in their natural, anatomic form. Our team has developed a novel whole-skull preparation technique utilizing formic acid to decalcify bone, conserving the integrity of the nasal cavity and permitting direct observation of neuroanatomy and pathology in the olfactory system of mice by routine immunohistochemistry (IHC). The useful applications of this technique are presented.

METHODOLOGY

Skull preparation and immunohistochemistry staining

Perfusion with PBS, then 10% formalin, by cardiac puncture at heart level. 4 μM thick sagittal cuts → Paraffin embedding → Fixation: skull removed and treated in 10% formalin X48 hrs. transfer to 70% EtOH → Decalcification: 12% formic acid for 3-5 days → Staining

Table 1: Quantification of reovirus infection in the olfactory epithelium of snca-null and hSNCAA53T mouse

<table>
<thead>
<tr>
<th>Mouse genotype</th>
<th>Reovirus infected cells per sinus</th>
<th>Healthy cells per sinus</th>
<th>Ratio infected/healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snca-null mouse</td>
<td>8 ± 3</td>
<td>128 ± 11</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>hSNCAA53T (n=4) mouse</td>
<td>19 ± 16</td>
<td>121 ± 24</td>
<td>0.16 ± 0.14</td>
</tr>
</tbody>
</table>

Step 1: sample 3 regions with the highest number of infected cells and 3 regions with primarily healthy cells.
Step 2: count infected and non-infected cells using ImageJ.

The severity of infection in the olfactory epithelium is represented by the ratio between reovirus-infected cells and healthy cells; the higher the ratio, the greater the infection. These preliminary results seem to indicate a relative “vulnerability” of the hSNCAA53T mouse to reovirus. This data needs to be validated using a larger sample size.

CONCLUSION

We have developed a novel immunohistochemical staining technique that allows direct visualization of the olfactory structures, in particular the olfactory receptor epithelium and cranial nerve-1 en route to the olfactory bulb.

Combining the most current knowledge and hypotheses on Parkinson disease pathogenesis, including Braak’s “two-hit” hypothesis by nasal and gastric entry of an unknown environmental pathogen, our protocol allows for the exploration of the interactions between environmental factors (including microbial agents) and genetic susceptibilities to late-onset neurological disorders in mammals.

ACKNOWLEDGEMENTS

This project was graciously funded by UROP and CIHR.

Dr. John Woulfe (Ottawa Hospital Research Institute) for valuable expertise in the recognition of mouse neuropathology.

Dr. Eric Robertson (University of Alabama at Birmingham) for the kind donation of tau-null transgenic mice.

Dr. Omar El Aghfif for kindly providing us with oligomeric SNCA-specific antibodies.

Ms. Dina Elieify for training and useful tips in IHC.

Ms. Jacqueline Tokarew for her help with the review of this poster.

SELECTED REFERENCES

1) Braak et al. Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. J Neural Trans 2003, 110:517-536.

CONTACT

Fanyi Meng, B.Sc. (Hons)
Email: fmeng026@uottawa.ca
Phone: 613-413-3363

Schlossmacher Lab
451 Smyth Road, RGN #1412
Ottawa, Ontario, Canada K1H 8M6
Tel: 613-562-5800 Ext 8184 (laboratory)