Microglia, Inflammation, and FTD

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Outline

- Why study inflammation in neurodegeneration?
- Why study PGRN in neuroinflammation?
- Ongoing research on PGRN
- Discussion
The Secret KILLER

The surprising link between inflammation and heart attacks, cancer, Alzheimer's, and other diseases.

READ THE STORY
Effect of NSAIDs on the Relative Risk of Developing AD

Normalized Risk

Adapted from In’t Veld et al. (2001), New England Journal of Medicine
Microglia Are Resident Immune Cells in the Brain

- Functionally related to periphery macrophages and other cells of monocytic lineage.
- Most likely derived from infiltrating hematopoietic or mesodermal cells during early development of CNS.
- Participate in immune surveillance of the CNS.
Pathogenic Processes in Neurodegenerative Diseases

- Alzheimer’s
- Parkinson’s
- FTD
- Huntington’s
- ALS
- ...

Neurodegeneration

Microglial Activation

Aggregates Accumulation
Different Protein Aggregates Accumulate Neurodegenerative Diseases

- Amyloid β
- Tau
- α-synuclein
- Huntingtin
- TDP-43
- ...

Aggregates Accumulation

Microglial activation

Neurodegeneration

Plaques

NFT
Chronic Inflammation in AD

Activated microglia

Amyloid Plaques
Activated Microglia Cluster Around Neuritic Plaques in AD Mouse Models
Different Protein Aggregates Accumulate Neurodegenerative Diseases

- Amyloid β
- Tau
- α-synuclein
- Huntingtin
- TDP-43
- ...

Degradation

Chronic Inflammation

Neurodegeneration

Plaques

NFT
Microglia: Friend or Foe?

Microglia as Foe

- Toxic factors
- Neuronal Damage

Microglia as Friend

- Aggregates clearance
- Protection
Outline

- Why study inflammation in neurodegeneration?
  - Why study PGRN in neuroinflammation in FTD?
- Ongoing research
- Discussions
Pathogenic Processes in Neurodegenerative Diseases

Aggregates Accumulation
- TDP-43
- Tau

Chronic Inflammation

Degradation

Neurodegeneration

- Alzheimer’s
- Parkinson’s
- FTD
- Huntington’s
- ALS
- …
Microgliosis and Astrogliosis in FTLD-U with PGRN Mutations
LETTERS

Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17

Matt Baker1*, Ian R. Mackenzie2*, Stuart M. Pickering-Brown3,4, Jennifer Gass1, Rosa Rademakers1, Caroline Lindholm5, Julie Snowden6, Jennifer Adamson7, A. Dessa Sadovnick8, Sara Rollinson9, Ashley Cannon1, Emily Dwosh1, David Neary1, Stacey Melquist4, Anna Richardson1, Dennis Dickson1, Zdenek Berger1, Jason Eriksen1, Todd Robinson1, Cynthia Zehr1, Chad A. Dickey1, Richard Crook1, Eileen McGowan1, David Mann1, Bradley Boeve2, Howard Feldman7 & Mike Hutton1

LETTERS

Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21

Marc Cruts1,2,3, Ilse Gijselinck1,2,3, Julie van der Zee1,2,3, Sebastiaan Engelborghs1,2,4, Hans Wils1,2,3, Daniel Picci1,2,3, Rosa Rademakers1,2,4, Rik Vandenberghhe1, Bart Dermaut1, Jean-Jacques Martin1,5, Cornelia van Duijn5,6, Karin Peeters7,8, Raf Sciot6, Patrick Santens5, Tim De Potter1,2,3, Maria Mattheijssens1,2,3, Marleen Van den Broeck1,2,3, Ivy Cuijt1,2,3, Krist'Vennekens1,2,3, Peter P. De Deyn5,6, Samir Kumar-Singh1,2,3 & Christine Van Broeckhoven1,2,3
What Is Progranulin (PGRN)?

- Composed of 7.5 tandem repeats of 6-Kda granulin peptide (Granulin A and Granulin B, a.s.k. Epithelin1/2)
- Involved in wound repair, tumorigenesis, and inflammatory responses
Null Mutations in PGRN Cause Tau-negative FTD
Mutations Are Associated with Reduction in the Levels of PRGN
Expression of Progranulin in Activated Microglia in FTD brains with PRGN Mutations

FTD with PRGN Mutation  AD
What is the role of PGRN in microglia?

Pathogenic?
Protective?
Bystander?
Outline

- Why study inflammation in neurodegeneration?
- Why study PGRN in neuroinflammation in FTD?
- Ongoing research
- Discussions
Hypothesis

FTD
(null mutation)

\[ \downarrow \]

PGRN

\[ \downarrow \]

Microglia activation

\[ \uparrow \]

Neurodegeneration

\[ \uparrow \]
A Cellular Model of Microglial Toxicity

- Primary
- Mimic neuron-glial interaction
- Cell type-specific genetic manipulation
The Cellular Model: Cortical Neuron-Glia Mixed Cultures

Neurons: ~20–30%
Astroglia: ~40–50%
Microglia: ~10–15%
Aβ Toxicity in Cortical Neuronal-Glial Cultures

MAP2 (neurons)

GFAP (astrocytes)

Aβ1-42
Microglia Mediate the Toxic Effects of Aβ in Mixed Cortical Cultures

Chen et al., JBC, 2005

Selective elimination of microglia
Aβ Activates NF-κB in Microglia and Astroglia

5X κB Enhancer Element

EGFP expression: NF-κB Activation

Microglia (cd11b)

Astroglia (GFAP)

Chen et al., JBC, 2005
Inhibition of NF-κB Signaling by Overexpressing IκBα-SR
Targeted Inhibition of NF-κB Signaling in Microglia Protects Against Aβ Toxicity

Adapted from Chen and Greene, J Mol Med., 2003

Chen et al., JBC, 2005
What is the role of PGRN in microglia?

Pathogenic?
Protective?
Bystander?
Progranulin Is Exclusively Localized in Microglia in Mixed Cortical Cultures
1. Is PGRN overexpression in microglia protective?
MCSF Promoter Targets Expression in Microglia in Cortical Cultures

**Diagram:**
- CMV
- HIV-1 flap
- MCSF
- EGFP
- WRE
- ΔU3

**Images:**
- EGFP
- Tomato Lectin
- Merge

**Text:**
MCSF (Macrophage Colony Stimulating Factor promoter)
Lenti-MCSF-PRGN Induces Progranulin Expression in Mixed Cortical Cultures

Conditioned Medium
Microglial-derived PRGN Protects Neurons Against Oligomeric Aβ-Induced Toxicity

MAP2-positive Neurons Survived 7PA2 CM

Lenti-MCSF   CTRL PGRNCTRL PRGN

Aβ

$P < 0.0005$  $P < 0.0005$
2. Does microglial PGRN suppress NF-κB activation?
NF-κB-dependent Expression of dEGFP in Microglial BV2 Cells

Chen et al., JBC, 2005
Transfecting PRGN in BV2 Cells: Electroporation With Amaxa Nucleofactor

1. BV2 microglia in serum-containing medium

2. Electroporation to transfict mock and PGRN DNA vectors at 1, 2, 4 μg

3. Changed to serum-free medium
Activation of NF-κB by Electroporation Is Associated with Downregulation of PRGN

Non-transfected

Mock (1.0 μg)  
Mock (2.0 μg)  
Mock (4.0 μg)

Conditioned Medium
Progranulin Overexpression Inhibits NF-κB Signaling
Electroporation-induced PRGN Overexpression

- Non-transfected
- Mock Transfection
- PGRN Transfection

Conditioned Medium
Progranulin Overexpression Inhibits NF-\(\kappa\)B Signaling in Microglia

\[ P < 0.0005 \]

N=11
## Confirmation of Progranulin Overexpression in LPS-stimulated BV2 Cells

<table>
<thead>
<tr>
<th>LPS</th>
<th>Mock</th>
<th>PGRN</th>
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</thead>
<tbody>
<tr>
<td>-</td>
<td>(1.0 µg)</td>
<td>(1.0 µg)</td>
</tr>
<tr>
<td>+</td>
<td>(2.0 µg)</td>
<td>(2.0 µg)</td>
</tr>
<tr>
<td></td>
<td>(4.0 µg)</td>
<td>(4.0 µg)</td>
</tr>
</tbody>
</table>

- | + | - | + |
- | + | - | + |

**Conditioned Medium**

[Image of Western blot with PGRN bands]
Recombinant Progranulin Induces Only a Modest Inhibition of LPS-induced NF-κB Signaling
Recombinant Progranulin Induces a Modest Inhibition of LPS-induced NF-κB Signaling

![Graph showing the effect of LPS on EGFP level with and without Progranulin (PGRN) treatment.](image-url)

Key:
- **Ctrl Bf**: Control Blanks
- **PGRN (20 μg/ml)**: Progranulin treatment

(N=3)
3. How is microglial PGRN regulated?

- Expression (transcription/translation)?
- Secretion/trafficking?
- Modifications/interactors?
Mutations Are Associated with Reduction in the Levels of PRGN
Expression of Progranulin in Activated Microglia in FTD brains with PRGN Mutations

FTD with PRGN Mutation

AD
Electroporation-induced PRGN Overexpression

Non-transfected  
Mock Transfection  
PGRN Transfection

3 hr 1 day 2 day 3 hr 1 day 2 day 3 hr 1 day 2 day

PGRN

Conditioned Medium
Viral Infections Reduced Levels of Secreted PGRN

Non-Treated

MCSF Ctrl Virus

MCSF PRGN Virus

75kDa

Conditioned Medium
Low Calcium Elevates Secreted PRGN Levels in Microglia
Ionomycin Downregulates Levels of Secreted PRGN (Mature Form) in Microglia
Low Calcium Elevates PRGN Levels in Electroporation

- Transfected
- PGRN
- Mock

<table>
<thead>
<tr>
<th>1.8mM Ca$^{2+}$</th>
<th>0.4mM Ca$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.8mM PGRN</td>
<td>0.4mM Mock</td>
</tr>
</tbody>
</table>

P < 0.01

EGFP Levels (NF-κB Activation)

(N=4)
Hypothesis

FTD  (null mutation)

PGRN

Microglia
activity

Neurodegeneration

Environmental Stress

[Ca²⁺]ᵢ
Points for Discussion

Conceptual
Technical
Translational
Points for Discussion

FTD (null mutation) → PGRN → Microglial NF-κB activation → Neurodegeneration

Stress → [Ca^{2+}]_i → ③

①

②

③
Points for Discussion

- PGRN
- Microglial NF-κB activation
- Neurodegeneration
- FTD (null mutation)
- Stress
- \([\text{Ca}^{2+}]_i\)
How does calcium homeostasis regulate PRGN? Transcriptional? Post-translational? Trafficking?

Yes

How does calcium homeostasis regulate PRGN? Transcriptional? Post-translational? Trafficking?

How is calcium homeostasis regulated by stress?

What are the signaling pathways downstream/upstream of the intracellular calcium?

Source of calcium matters? Intracellular calcium store? Extracellular calcium influx?
Decrease of Progranulin by ionomycin is not transcriptionally regulated

(N=2)
Translational

✓ How to promote PGRN levels by modulating calcium homeostasis?