The ryanodine receptor is a novel target for Bcl-2

Tim Vervliet
26/10/2012

Promoter: Geert Bultynck
Co-promoter: Jan Parys
Laboratory for Molecular and Cellular Signaling
### \( \text{IP}_3 \text{Rs} \leftrightarrow \text{RyRs} \)

<table>
<thead>
<tr>
<th></th>
<th>( \text{IP}_3 \text{R} )</th>
<th>( \text{RyR} )</th>
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</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>±300kDa</td>
<td>±500kDa</td>
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<tr>
<td>Number of isoforms</td>
<td>3</td>
<td>3</td>
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<tr>
<td>Expression</td>
<td>All cells</td>
<td>High expression in specific cell types: skeletal muscle, heart, brain...</td>
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<tr>
<td>Functions</td>
<td>Cell survival, differentiation, proliferation, autophagy, cell death...</td>
<td>Muscle contraction Neuronal signaling: LTP, LTD...</td>
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</tbody>
</table>
The Bcl-2-protein family

- Multidomain anti-apoptotic: BCL2, BCL-XL, MCL1, BCL-w and BFL1
  - BH4 - BH3 - BH1 - BH2 - TM

- Multidomain pro-apoptotic: BAX, BAK and BOK
  - BH3 - BH1 - BH2 - TM

- BH3-only pro-apoptotic: Activators: BID, BIM and PUMA(?)
  - Sensitizers: BAD, BIK, NOXA, BMF and HRK
  - BH3

Letai, 2008, Nature Reviews Cancer
The Bcl-2-protein family

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Hydrophobic cleft

BH3 domain Beclin1

Letai, 2008, Nature Reviews Cancer
The Bcl-2-protein family

**Hydrophobic cleft**

BH3 domain Beclin1

Letai, 2008, Nature Reviews Cancer
Why study the Bcl-2/RyR interaction?

Bcl-2-binding site IP₃R

<table>
<thead>
<tr>
<th></th>
<th>IP3R1</th>
<th>IP3R2</th>
<th>IP3R3</th>
<th>RyR 1</th>
<th>RyR 2</th>
<th>RyR 3</th>
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Upregulation of Bcl-2 in HEK RyR3 cells
Full-size interaction

HEK RyR3 cells

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<thead>
<tr>
<th>RyR antibody</th>
<th>+</th>
<th>−</th>
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<tr>
<td>IgG</td>
<td>−</td>
<td>+</td>
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anti-RyR

anti-Flag

3xFlag        Bcl-2
Full-size interaction

HEK RyR3 cells

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<tr>
<th>RyR antibody IgG</th>
<th>+</th>
<th>-</th>
<th>+</th>
<th>+</th>
<th>-</th>
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<th>+</th>
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<td>anti-RyR</td>
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<td>anti-Flag</td>
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<td>Endogenous Bcl-2</td>
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anti-RyR
anti-Bcl-2
Bcl-2 interacts with the RyR during differentiation of C2C12 cells
Bcl-2 interacts with RyR in hippocampal rat-brain lysates
Bcl-2 inhibits caffeine-induced Ca^{2+} release
Cell biological role in cell fate: Autophagy?

- HEK RyR3 cells show increased basal levels of LC3-II
Modulating RyR3 activity influences autophagy in HEK RyR3 cells

- RyR3 activity influences LC3-II formation in HEK RyR3 cells
Conclusions

• Bcl-2 interacts with the RyR
• Interaction of Bcl-2 with RyR3 inhibits caffeine-induced Ca^{2+} release
• Role in autophagy?
Acknowledgements

Laboratory for Molecular and Cellular Signaling:
Giovanni Monaco
Santeri Kiviluoto
Kirsten Welkenhuyzen
Tomas Luyten
Humbert De Smedt
Ludwig Missiaen
Jan Parys
Geert Bultynck

Physiology group UGENT:
Elke Decrock
Luc Leybaert

Laboratory of Intracellular Ion Channels Bratislava:
Zuzana Tomaskova
Karol Ondrias

Donders Institute for Brain, Cognition and Behaviour Nijmegen:
Nael Nadif Kasri

Molecular Medicine Section university of Sienna:
Vincenzo Sorrentino