Lipid Rafts

- A **lipid raft** is a cholesterol-enriched microdomain in cell membranes.
- They contain: cholesterol, glycolipids, sphingolipids, and proteins.
- Lipid rafts are involved in molecular trafficking, immune system functions, as well as signaling transduction.
- They can be stimulated to cluster by: tumor necrosis factor α, Fas ligand, endostatin, *et al.*
NADPH Oxidase-Derived Superoxide in the Kidney

- NAD(P)H oxidase is a membrane associated enzyme that generate superoxide
- 5 subunits: p47, p67, p40, p22, Nox isoform, and Rac
- one of major enzymes responsible for $O_2^-$ production in the kidney under physiological conditions

memorias.ioc.fiocruz.br/100(Suppl)/5253NO01.html
Activation Mechanisms of NADPH Oxidase

- P47 Translocation
- Rac GTPase activity

Hyperhomocysteinemia

Hyperhomocysteinemia is a critical pathogenic factor in the progression of ESRD and in the development of cardiovascular complications related to ESRD.

Hyperhomocysteinemia occurs in 85%-100% of patients in ESRD.

Hyperhomocysteinemia levels:
- < 10 µmol/L: Normal
- 15-30 µmol/L: Mild
- 30-60 µmol/L: Moderate
- > 80 µmol/L: Severe
Background

L-Hcys → Ceramide synthase
  ↓ de novo synthesis
  ↓ Activation

Ceramide

Rac-GTP → NAD(P)H oxidase

Superoxide

Apocynin or DPI

Glomerular Injury
  ↓ MMP-1 activity
  ↑ TIMP-1

Myriocin or Fumonisin B₁

Reduction
Background

Fas Ligand

\[ \downarrow \]

Lipid raft formation

\[ \downarrow \]

Redox platform formation on the lipid raft

\[ \downarrow \]

p47 translocation to lipid raft

Gp91 aggregation on the lipid raft

Rac GTPase translocation to lipid raft

**Question:**

Whether redox signaling platforms formed on the lipid raft are involved in the regulation of Hcys-induced NADPH oxidase activity?
Methods: Isolation of lipid rafts

Gradient centrifugation

- 5% OptiPrep Density Gradient Medium
- 30% OptiPrep Density Gradient Medium
- 40% OptiPrep Density Gradient Medium
Methods: Western Blot

• Separation of proteins via electrophoresis
• Proteins in gel are transferred to nitrocellulose membrane
• Membrane is probed with antibodies
Results

Characterization of target enzymes in glomerular endothelial cells

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Molecular Weight</th>
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<tr>
<td>49kDa-p47</td>
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<td>40kDa-p67</td>
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<tr>
<td>58kDa-gp91</td>
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<td>26kDa-p22</td>
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<tr>
<td>57kDa-Nox-4</td>
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<td>21kDa-Rac</td>
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<td>35Kda-Timp-1</td>
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<td>80kDa-Vav sigma</td>
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<td>95kDa-pc2</td>
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Control    | Hcys
Results

Isolation of lipid raft from glomerular endothelial cells

control
- Flutilin-1 lipid raft marker
- Rac

Hcys
- Flutilin-1
- Rac

C16-ceramide
- Flutilin-1
- Rac
Results

P47

- Control
- Hcys
- C16
- Caveolin-1

Lipid raft

Relative distribution of p47 on lipid raft

control  Hcys  C16-ceramide
Results

**Rac 2**nd

- Control
- Hcys
- C16

Lipid raft

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Relative distribution of Rac on lipid raft

control Hcys C16-ceramide

0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8
Conclusion

• Possible that lipid raft-redox platform is involved in Hcys induced NADPH oxidase by p47 translocation
• Need more data to confirm these findings
Acknowledgment

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