Loss of HS1 inhibits neutrophil extravasation during inflammation via disturbed PKA signaling

IV. Immunology Summit, Houston, 29.09.2015

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Domain structure of hematopoietic cell-specific lyn substrate (HS1)

Billadeau & Burkhardt, Traffic, 2006
HS1 is best studied at the immune synapse

Billadeau & Burkhardt, Traffic, 2006
Summary of known HS1 functions

- can bind F-actin and actin nucleators such as Arp2/3 complex and WASP
- coordinates molecular components required for protrusion formation
- differential tyr-phosphorylation mediates adhesion to integrins and chemotaxis in NK cells
- is recruited to the immune synapse upon TCR engagement
- recruits Vav-1 to the IS and controls activation of cdc42 and Rac-1 upon TCR engagement
- HS1-KO mice are viable but show a defect in lymphocyte clonal expansion and B-cell homing

HS1 is involved in the regulation of granulopoeisis and it interacts with Arp2/3 in neutrophils to mediate chemotaxis
Cortactin deficiency affects leukocyte adhesion and transendothelial migration

Schnoor et al., 2011, J. Exp. Med., 208, 1721-35
General Questions

Does HS1 influence leukocyte extravasation \textit{in vivo}?  

Which steps of the cascade are affected?  

Which mechanisms are responsible for altered extravasation?
The leukocyte extravasation cascade

- Tethering
- Rolling
- Firm Adhesion
- Intraluminal Crawling
- Transendothelial Migration

Vascular Endothelium

Selectins (E, P)
Selectin Ligand (e.g., PSGL-1)
Chemoattractant Receptor
- Chemoattractant
- $\alpha_4\beta_2$ (LFA-1)
- $\alpha_4\beta_2$ (Mac-1)
- ICAM-1

Tissue Injury

Chemotaxis

McDonald & Kubes, J Mol Med, 2011
Intravital microscopy of inflamed cremaster venules
Intravital microscopy of inflamed cremaster venules
HS1-deficiency affects KC-induced leukocyte extravasation

rolling velocity [µm/s]  adhesion [x10^2 cells/mm^2]

transmigration [cells]
HS1-deficiency does neither affect vessel morphology in the cremaster nor surface expression of adhesion molecules.
Reduced adhesion is not due to defective signaling mediated by PLC, Akt, Erk or p38 MAPK

<table>
<thead>
<tr>
<th>phospho-specific signal</th>
<th>total lysate</th>
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<tbody>
<tr>
<td><strong>WT</strong></td>
<td><strong>KO</strong></td>
</tr>
<tr>
<td>-</td>
<td>- KC</td>
</tr>
<tr>
<td>150</td>
<td>150</td>
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<tr>
<td>75</td>
<td>75</td>
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<tr>
<td>37</td>
<td>37</td>
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<td>37</td>
<td>37</td>
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</tbody>
</table>

- PLCγ
- Akt
- ERK
- p38
- HS1
PKA is involved in mediating HS1-dependent effects on transmigration

In vivo imaging reveals PKA regulation of ERK activity during neutrophil recruitment to inflamed intestines. Mizuno et al. 19.May.2014

PKA activation increases TEM only in WT PMN

PKA inhibition reduces KC-triggered TEM only in WT PMN
HS1 is not a direct PKA substrate

<table>
<thead>
<tr>
<th>Pre-IgG control</th>
<th>HS1-IP</th>
<th>VASP-IP</th>
</tr>
</thead>
<tbody>
<tr>
<td>UT 15</td>
<td>UT 15</td>
<td>UT 15</td>
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</tbody>
</table>

WB: PKA substrate consensus AB

80 kDA —

50 kDA —

p-HS1

p-VASP

HS1 level

VASP level
PKA is functional in the absence of HS1

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>KO</th>
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<tbody>
<tr>
<td>N6</td>
<td></td>
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</table>

- HS1 (80 kDa)
- p-VASP S\textsuperscript{157} (49 kDa)
- tubulin (52 kDa)

KC triggers PKA-mediated VASP phosphorylation

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<tr>
<th></th>
<th>untr.</th>
<th>KC</th>
<th>N6</th>
<th>Rp</th>
<th>Rp+KC</th>
</tr>
</thead>
</table>

- p-VASP S\textsuperscript{157} (49 kDa)
- VASP (46/49 kDa)
Phosphorylated HS1 and VASP co-precipitate

<table>
<thead>
<tr>
<th></th>
<th>WCL</th>
<th>HS1-IP</th>
<th>ctrl-IP</th>
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<tbody>
<tr>
<td>IL-8:</td>
<td>-</td>
<td>5</td>
<td>15</td>
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**VASP**

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<th>WCL</th>
<th>HS1-IP</th>
<th>ctrl-IP</th>
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**HS1**

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<th>HS1-IP</th>
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**S^{157}\text{-VASP}**

|       |       |        |         |

**p-tyr-HS1 (4G10)**

|       |       |        |         |
PKA inhibition prevents chemokine-induced phosphorylation of VASP and interaction with HS1

<table>
<thead>
<tr>
<th>Conditions</th>
<th>IL-8</th>
<th>PKA-Inhib</th>
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<tbody>
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<tr>
<td>- - +</td>
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</tr>
</tbody>
</table>

- **HS1_WCL (80 kDa)**
- **HS1_IP (80 kDa)**
- **S157-VASP_HS1 IP (49 kDa)**
- **S157-VASP_WCL (49 kDa)**
- **S157-VASP_ctrl IP (49 kDa)**

Heavy chain
Possible function of HS1 in the regulation of leukocyte transmigration

Chemokine (KC or IL-8) receptor

PMN

Chemokine (KC or IL-8)

PMN

ICAM-1 endothelial cell

PMN

firm adhesion and support of TEM

activation of LFA-1 Clustering?

GEF/GAP?

activation of Rap1

p-VASP/p-HS1

p-HS1

p-VASP

VASP

PKA

cAMP

SFK
Acknowledgement

MPI Münster
Dietmar Vestweber

TU Braunschweig
Klemens Rottner

Funding

CINVESTAV, Molecular Biomedicine
Hilda Vargas,
Alí Citalán, Alex García,
Martha Velázquez, Sandra Chánez,
Karla Castro, Omar Rodríguez
Vascular and Mucosal Pathobiology Special Interest Subgroup (VAMP-SIG) of the American Society of Investigative Pathology (ASIP)

Please visit: http://www.asip.org/SIGs/vmp/ or http://www.facebook.com/asipvamp

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3-year membership term for the price of 1 year for trainees

Please visit: www.leukocytebiology.org

For more information please contact me: mschnoor@cinvestav.mx
HS1 deficiency does not cause altered cell numbers in peripheral blood

<table>
<thead>
<tr>
<th></th>
<th>mean [WT]</th>
<th>SD</th>
<th>mean [KO]</th>
<th>SD T-Test (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cells [K/µl]</td>
<td>6.91064</td>
<td>1.0471219</td>
<td>7.55154</td>
<td>1.15649258</td>
</tr>
<tr>
<td>PMNs [K/µl]</td>
<td>1.32486</td>
<td>0.36698775</td>
<td>1.50042</td>
<td>0.60491002</td>
</tr>
<tr>
<td>Lymphocytes [K/µl]</td>
<td>4.80916</td>
<td>0.85250537</td>
<td>5.5869</td>
<td>1.05215898</td>
</tr>
<tr>
<td>Monocytes [K/µl]</td>
<td>0.17318</td>
<td>0.01581398</td>
<td>0.17088</td>
<td>0.06742749</td>
</tr>
<tr>
<td>Red blood cells [M/µl]</td>
<td>11.32396</td>
<td>1.13459566</td>
<td>11.5424</td>
<td>1.20527106</td>
</tr>
<tr>
<td>Platelets [K/µl]</td>
<td>990.244</td>
<td>144.671792</td>
<td>945.656</td>
<td>268.108192</td>
</tr>
<tr>
<td>Hemoglobin [g/dl]</td>
<td>16.31066</td>
<td>1.15588788</td>
<td>15.38868</td>
<td>2.25486595</td>
</tr>
</tbody>
</table>
KC triggers adhesion and transmigration of WT PMNs much stronger compared to KO
HS1-KO PMNs show the same morphology under resting conditions but unorganized protrusion formation after activation.