RED CELLS IN NEUROACANTHOCYTOSIS

Alessandro Matte’, Francesca Lupo, Lucia De Franceschi

• Alessandro Mattè
  nothing to disclose
Learning Objectives

• To recognize that acanthocytes are a marker of neuroacanthocytosis (NA), a set of hereditary neurodegenerative diseases affecting basal ganglia;

• To understand that different factors such as Lyn activation, targeting band-3, are involved in instability of ChAc RBC membrane-skeletal network;

• To understand that perturbation of band-3 based multi-protein complexes, is involved in abnormal RBC features in ChAc, MLS and HDL2 diseases.
Neuroacanthocytosis (NA)

NA is a heterogeneous group of hereditary neurodegenerative disorders characterized by:

- neurodegeneration of basal ganglia,
- peripheral neuromuscular manifestations,
- seizures and neuropsychiatric features,
- acanthocytosis

De Franceschi L et al 21: 201, 2014
Acanthocytes are abnormal red cells with thorn-like protrusions and represent one of the biological hallmarks of Neuroacanthocytosis (NA).
Abnormal Ultrasctrure Membrane-Skeleton Organization Has Been Documented in Acanthocytes from NA Patients

Terada N 101: 25, 1999; Mohandas N 112: 3939, 2008
Red Cell Membrane Organization is Based on Band 3 Anchoring Multiprotein Complexes

De Franceschi L et al. Curr Opinion in Hematol 2014
Red Cell Membrane Organization and Tyrosine Protein Phosphorylation

- Changes in Tyrosine (Tyr-)phosphorylation state of RBC membrane proteins might affect RBC membrane stability.

- RBC treated with vanadate to block phosphatases show increased Tyr-phosphorylation of band 3 and generation of acanthocyte-like shape RBCs.

Tyr-phosphorylation of band 3 is mediated by a cascade tyrosine kinases involving Syk as primary phosphorylation followed by translocation of Lyn, a Src family kinase.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mode of inheritance</th>
<th>Gene (location)</th>
<th>Protein product</th>
<th>Acanthocytes/anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChAc</td>
<td>AR</td>
<td>VPS13A (9q21)</td>
<td>Chorein</td>
<td>++++/No anemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25-60% Acanthocytes</td>
</tr>
<tr>
<td>MLS</td>
<td>X-linked</td>
<td>XK (Xp21)</td>
<td>XK protein</td>
<td>++++/Mild compensated hemolytic anemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25-60% Acanthocytes</td>
</tr>
<tr>
<td>HDL2</td>
<td>AD</td>
<td>JPH3 (16q24.3)</td>
<td>Junctophilin-3</td>
<td>++/No anemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30-35% Acanthocytes</td>
</tr>
<tr>
<td>PKAN</td>
<td>AR</td>
<td>PANK2 (20p.13)</td>
<td>Pantothenate kinase 2</td>
<td>±/No anemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3-10% Acanthocytes</td>
</tr>
</tbody>
</table>

De Franceschi L et al 21: 201, 2014
Neuroacanthocytosis (NA)

- Chorea-acanthocytosis (ChAc)
- McLeod Syndrome (MLS)
- HDL-2
- PANK

De Franceschi L et al 21: 201, 2014
Acanthocytes and Chorea-Acanthocytosis (ChAc): the Band 3 Connection

• Chorein is the protein encoded by the VPS13A gene involved in chorea-acanthocytosis (ChAc).

• Chorein has been detected in mature healthy RBCs but its role in RBC homestasis is unknown.

• In ChAc RBCs:
  – Sub-population of dense RBCs, containing acanthocytes
  – Normal membrane lipid composition
  – Increased N-glutamyl lysine isopeptide linking band 3 to ankyrin

Proteomic Analysis of RBC Membrane Revealed Differences in Post-Translation Profile of RBC Membrane Proteins
Tyr-phosphoproteome of ChAc RBC Membrane Differs from Healthy RBCs, Involving Band 3

De Franceschi L Blood 118: 5652, 2011
In ChAc RBCs Lyn Membrane Association is Increased and Independent from Syk Primary Phosphorylation.
Computational Analysis of Tyr-Phosphoproteome from ChAc RBCs Identified 14 Tyr-Kinases Highly Functionally Connected
RBC as Cell Model: Lyn a Bridge Between erythroid cells and neuronal cells

• Lyn and Fyn is involved in cell activities of several brain areas

• Lyn and Fyn up-regulate NMDA receptor activity in the central nervous system

• Genetic ablation of Fyn and Lyn results in behaviour abnormalities in mice, possibly through Tyr-phosphorylation of key substrates such as neurotransmitter receptors

Neuroacanthocytosis (NA)

Chorea-acanthocytosis (ChAc)

McLeod Syndrome (MLS)

HDL-2

PANK

De Franceschi L et al 21: 201, 2014
Mcleod Syndrome (MLS)

- MLS is a X-linked form of NA, involving the XK protein (444 aminoacid residues, containing the Kx antigen)

- The XK protein is predicted to have 10 transmembrane domains with structural characteristics of a ion transporter

- In RBC membrane, the XK protein is covalently linked to the Kell glycoprotein and is part of the band 3- 4.1 junctional complex, anchoring the membrane with the cytoskeleton

MLS and RBCs

- In **MLS**, truncation of the **XK protein** is associated with **reduced expression of the Kell blood group antigen**

- Electron microscopy analysis of **MLS RBCs** reveals heterogeneous distribution of RBC membrane skeleton

- **MLS RBCs** show reduced resistance to mechanical stress, increased RBC density and reduced cell K⁺ content

Abnormalities in Tyr-phosphorylation Proteome Characterize MLS RBCs

• Preliminary proteomic data on MLS RBCs indicate differences in membrane Tyr-phosphorylation pattern compared to healthy RBCs.

• In MLS RBCs, proteins identified with different Tyr-phosphorylation state are: band 3, ankyrin and protein 4.1

De Franceschi L 7: e31015, 2012
In MLS, different factors might contribute to MLS RBC membrane instability and to the generation of acanthocytes:

- Reduction in XK Kell complex
- Abnormal Tyr-phosphorylation state, affecting protein-protein interactions
- Destabilization of membrane-cystoskeleton network

Neuroacanthocytosis (NA)

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De Franceschi L et al 21: 201, 2014
Huntington Disease like-2 (HDL2)

- HDL2 is an autosomal dominant form of NA, resembling Huntington disease

- HDL2 is caused by a mutation on junctophilin-3 (JPH3)

- HDL2 has been identified only in African descent patients

Walker RH et al. 58: 1031, 2002; De Franceschi L et al 21: 201, 2014
HDL2 and RBCs

• Acanthocytes have been observed in extremely variable percentage: 20-65%

• HDL2 RBCs show:
  – band 3 breakdown products
  – Increased release of erythroid vesicles
  – Increased membrane association of small G-proteins

• These data suggest abnormalities in RBC membrane organization in HDL2

Walker RH et al. 58: 1031, 2002; De Franceschi L et al 21: 201, 2014
# Mouse Model of HDL2

<table>
<thead>
<tr>
<th>HDL2</th>
<th>Mice JPH3&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>No or mild neurological phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mice JPH3&lt;sup&gt;−/−&lt;/sup&gt; JPH4&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Cognitive and motor deficiencies</td>
</tr>
<tr>
<td></td>
<td>Mice BAC-HDL2</td>
<td>Age dependent motor deficiency</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neuronal phenotype of HDL2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No data on RBC features</td>
</tr>
</tbody>
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Walker RH et al. 58: 1031, 2002; De Franceschi L et al 21: 201, 2014
Moriguchi S et al. 103: 10811, 2006; Wilburn B et al. 70: 427, 2011
Neuroacanthocytosis (NA)

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De Franceschi L et al 21: 201, 2014
Panthotenate Kinase Associated Neurodegeneration (PKAN)

- PKAN is an autosomic recessive disorder, characterized by defect in panthothenate kinase 2 expression and activity.

- PKAN2 localizes in mitochondria and is a key enzyme in the biosynthesis of coenzyme A (CoA).

- Acanthocytes have been detected in 3-10% of the PKAN subjects.

- PKAN2 patients show brain iron overload in the globus pallidus, resulting in the eye of tiger pattern on magnetic resonance.

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PKAN and RBCs

Recent studies on PKAN RBCs have shown:

– Reduced ability of PKAN acanthocytes to form drug induced endovesicles compared to normal shaped PKAN RBCs

– Reduced ability of PKAN RBCs to undergo extreme deformation through a spleen-like device compared to healthy RBCs

– No differences in time of deformability and relaxation of PKAN RBCs in microfluidic device mimicking microcirculation share stress compared to healthy controls

De Franceschi L et al 21: 201, 2014; Siegel C et al. 8: e76715, 2013; Cluitmans JC et al. 10: e0125580, 2015
Conclusions

• In NA the mechanisms underlying neuronal degeneration of basal ganglia and acanthocyte formation is still under investigation

• The recent progresses on characterization of NA RBC features link perturbation of signal transduction pathway to weakening of band 3 based multi-protein complexes, bridging the membrane to the RBC cytoskeleton.

• Since RBC membrane contains proteins present in other cell systems such as neurons, the specific and characteristic association of acanthocytosis with neurodegeneration suggests RBCs as promising target for future mechanistic and functional studies in NA.
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<thead>
<tr>
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