CHARACTERIZATION OF SCD BI-PHASIC RESPIRATORY MANIFESTATIONS TOWARD THERAPEUTIC STRATEGIES

Marie Trudel, PhD

Molecular Genetics and Development
Institut de recherches cliniques de Montréal - IRCM
Université de Montréal
RED BLOOD CELLS IN SICKLE CELL DISEASE

- 1910 James Herrick describes sickle RBC
- RBC sickles as a consequence of hemoglobin, the most abundant protein, polymerization or chain due to a hereditary mutation in the gene producing hemoglobin.
- Anemia results from destruction of the abnormal or sickle RBC
HUMAN SICKLE CELL DISEASE CLINICAL MANIFESTATIONS

- **Respiratory system** (defective respiratory and pulmonary function):
  - ACS is the 1st cause of death in early childhood to adulthood
  - Chronic lung disease: changes in lung function, pulmonary hypertension
  - Impaired exercise capacity

- **Hematology**
  - Hemoglobin polymerization ➔ RBC sickling ➔ Irreversible sickled cells/ISC
  - Anemia
  - Leucocyte activation and inflammation

- **Vascular to Systemic complications**
  - Vasooclusions, infarctions, generalized congestion in some organs
  - Stroke Neuronal: pain, retinal degeneration
  - Kidney: functional defects and glomerulopathy
  - Priapism
  - Ulcers: skin, leg
  - Osteonecrosis
LIFESPAN OF SCD POPULATION IN 1979

Age at death by age group

Percentage of deaths

<1  1-4  10-14  20-24  35-44  55-64
Toward permanent treatments for SCD

Certain patients with SCD and thalassemia are candidates for gene therapy using autologous (their own) hematopoietic stem cells.

Development, optimized and purification of lentiviral vectors for permanent correction via gene therapy

Stem cell engraftment implicates myeloablative conditioning.

Clinical trials in Thailand, France, USA, Australia: 20 subjects

Most thalassemia major patients become transfusion-independent

One SCD reduced transfusion – no hospitalization or acute episode

Promising future technology are by genome editing using CRISPR-Cas9

SCD skin $\rightarrow$ iPS $\xrightarrow{\text{CRISPR-Cas9}}$ HSC $\rightarrow$ transfer to recipient

to reactivate fetal Hb or directly correct the mutation in the gene of HbS

in vivo efficiency and efficacy risk and safety
NEWBORN SAD MICE

Evidence of palor - anemia and smaller size
Hematology

- Anemia (severe at pre-weaning age – mild adulthood)
- *in vivo* Hb polymer and RBC sickling
- No evidence of globin chain imbalance and normal MCH or thalassemia

Systemic complications

- Vasooclusions with thrombosis, generalized congestion
- Infarctions in several organs: kidneys, liver, penile/priapism, myocardium, lung, spleen
- Renal glomerular hyperthrophy/papillary necrosis and altered function
- Liver kupffer cell erythrophagocytosis
- Ulcers skin
- Retinal degeneration
- Premature death
SAD COHORTS REDUCED SURVIVAL

- At P3 35% of mice are SAD positive theoretical predicted rate of SAD genotype is 50% on homogenous genetic background.

- Two groups of SAD mice at P3 (n=88):
  - One cohort consists of 43% of SAD mice with body weight >20% below WT ➔ SAD>20
  - Second cohort of 57% of SAD mice with body weight <20% below WT ➔ SAD<20

- 26% of SAD>20 mice die between P3-P6
Whole-body plethysmographic measurements of newborn P3 SAD mice with severe phenotype have unstable breathing and apneas (arrows).
Population of young P3 SAD mice have unstable breathing. Normal tidal volume suggests pulmonary obstructive pattern as in children with SCD.

Apneas in SAD/SCD will induce hypoxia and hypercapnia state and in turn will increase RBC sickling and vasoocclusions.
SAD P7 MICE EXHIBIT ALTERED BREATHING IN NORMOXIA AND HYPOXIA

• Whole-body plethysmography under hypoxic conditions accentuate abnormal breathing in P7 SAD mice.
• After 3 minutes, SAD displays apneas, stroke-like activity followed by gasping.
Under hypoxia, both SAD cohorts at P7 from whole-body plethysmography show abnormal breathing, stroke/seizure and lethality that is more severe in the SAD$^{>20}$. 
In normoxia, SAD>20 lungs show microvascular occlusions, infarcts and congestion, small hemorrhages with septal and alveolar thickening with marked infiltrates - inflammation as defined for ACS diagnostic.

Under hypoxia, severity is exacerbated.
SAD PHENOTYPE IN THE PRE-WEANING PERIOD

57% of SAD pups (SAD<20) show
- Slightly lower body weight (<20% less than WT)
- Moderately increased apnea in room air and hypoxia
- Viable into adulthood

43% of SAD pups (SAD>20) show
- Lower body weight
- Impaired respiratory rhythmogenesis
- Apnea long periods are consistent with high risk for nocturnal oxygen desaturation and obstructive sleep apnea in childhood SCD
- Obstructive pulmonary pattern
- Pulmonary infiltrates and vascular congestion
- ACS-like
- Stroke - terminal apneas in response to hypoxic challenge
- Acute respiratory failure and lethality prior to weaning
EVALUATION OF ADULT SAD MICE BREATHING IN NORMOXIA

From plethysmography analysis, approximately half of the adult SAD mice at 7-8 months of age have regular low amplitude, high respiratory frequency - tachypnea
SAD ADULT COHORTS

Two groups of SAD mice at >6 mo of age (n=77):

• First cohort consists of 48% of SAD mice with tachypnea \(\rightarrow\) SAD\textsuperscript{T}

• Second cohort of 52% of SAD mice had no tachypnea \(\rightarrow\) SAD\textsuperscript{NT}
Population of adult SAD\textsuperscript{T} mice whole-body plethysmography show increased respiratory frequency (F\textsubscript{R}), decreased tidal volume (V\textsubscript{T}, ml/gram) and increased minute volume (V\textsubscript{E}) consistent with a typical restrictive pattern.

Population of adult SAD\textsuperscript{NT} appears indistinguishable from WT.
• Population of adult SAD mouse displays apneas and strokes/seizures with a milder phenotype in the adult SAD<sup>NT</sup> population.
• ~15% of SAD<sup>T</sup>mice died following hypoxic exposure.
Adult SAD\textsuperscript{T} and SAD\textsuperscript{NT} mice have decreased in exercise tolerance revealing a chronic restrictive lung disease.

→ Similar to SCD patients dyspnea, restrictive lung defect and exercise limitations.
IN VIVO PHYSIOLOGIC ANALYSIS OF ADULT SAD LUNG MALFUNCTION

SAD mice with tachypnea have decreased lung capacity - compliance and increased lung elasticity → a chronic restrictive pulmonary pattern

SCD patients with restrictive lung defects are typically dyspneic with increased overall respiratory center drive from increased lung elasticity and adopt a tightly constrained breathing pattern to reduce dyspnea.
SAD MICE DEVELOP BIVENTRICULAR HYPERTROPHY AND PULMONARY HYPERTENSION

- Echography analysis showed decreased pulmonary artery acceleration time (PAAT) in most adult SAD$^T$ mice indicating pulmonary hypertension.
- Increased right ventricle internal diameter and cross-sectional area and increased mass of left ventricle provide evidence for right and left ventricular hypertrophy.
**ADULT SAD LUNG PHYSIOPATHOLOGY**

<table>
<thead>
<tr>
<th>Normoxia</th>
<th>Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td></td>
</tr>
<tr>
<td>SAD$^{NT}$</td>
<td></td>
</tr>
<tr>
<td>SAD$^{T}$</td>
<td></td>
</tr>
</tbody>
</table>

- Adult SAD$^{T}$ vascular congestion, dilated pulmonary vessels, mild septal thickening with infiltrates correlates with lung dysfunction and ACS
- Development of ~2-4 fibrin/platelet thrombi per 0.5cm$^2$ section, 24h after hypoxia
**SUMMARY OF SAD LONGITUDINAL PULMONARY COMPREHENSIVE STUDY**

<table>
<thead>
<tr>
<th>SAD mice</th>
<th>SCD patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1st phase:</strong> young and pre-weaning</td>
<td>Children</td>
</tr>
<tr>
<td>• Apnea</td>
<td>• Obstructive sleep apnea</td>
</tr>
<tr>
<td>• Obstructive pulmonary disease</td>
<td>• Nocturnal oxygen desaturation</td>
</tr>
<tr>
<td>• ACS-like episode</td>
<td>• Obstructive pulmonary disease</td>
</tr>
<tr>
<td></td>
<td>• ACS</td>
</tr>
<tr>
<td><strong>2nd phase:</strong> adult</td>
<td>Adult</td>
</tr>
<tr>
<td>• Chronic restrictive pulmonary disease</td>
<td>• Chronic restrictive pulmonary disease</td>
</tr>
<tr>
<td>• ACS-like episode</td>
<td>• ACS</td>
</tr>
<tr>
<td>• Pulmonary hypertension</td>
<td>• Pulmonary hypertension</td>
</tr>
</tbody>
</table>

ACS-like episode: SAD housing in SPF facility - ACS mechanism is independent of infections or fever etc

→ SAD/SCD mouse model reproduces in distinct phases the hallmarks of respiratory pathophysiology as in human SCD.
Outcomes and Future plans

Outcome on clinical practice: translational findings

→ Restrictive pattern of lung function warrant systematic monitoring of adult SCD patients for disease progression by non-invasive methodology

Research program on the mechanisms

- Obstructive syndrome in SCD probably starts around rather than inside the airways: possibly in small peri-alveolar and/or peri-bronchiolar from vascular occlusions?
- Chronic restrictive pulmonary disease in SCD: results possibly from obstruction of the airways due to the inflammatory process? starts in the airways.

Preclinical Studies

→ Targeted therapies and therapeutic intervention to evaluate efficacy of specific treatment in distinct type- and phase of specific SAD/SCD cardiorespiratory pathophysiologies

Research – clinical questions

- In human, do we observe a bimodal appearance of SCD respiratory symptoms for some patients as detected in mice?
- Are drugs used to treat typical allergic asthma or inflammation suitable or efficient to prevent the obstructive syndrome in SCD?
- Can we identify and repurpose drugs to prevent ACS?
COLLABORATORS

Dept Pediatrics and Physiology, WCHRI, U. of Alberta
• Joanna MacLean, MD, PhD
• Jun Ren, PhD
• John J. Greer, PhD

Molecular Genetics and Development, IRCM
• Josepha-Clara Sedzro, graduate student

Funding Support
Women and Children’s Health Research Institute, University of Alberta
Canadian Institute of Health Research (CIHR)
Canadian Blood Services
### CRITICAL PHASES OF SAD LUNG PATHOPHYSIOLOGY

<table>
<thead>
<tr>
<th>P0-20 pre-weaning</th>
<th>7-8 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>43% of SAD pups</strong></td>
<td><strong>48% of SAD survivors</strong></td>
</tr>
<tr>
<td>- Lower body weight</td>
<td>- Tachpnea</td>
</tr>
<tr>
<td>- Apneas, impaired</td>
<td>- Pulmonary hypertension-</td>
</tr>
<tr>
<td>respiratory rhythm</td>
<td>heart hypertrophy</td>
</tr>
<tr>
<td>- Pulmonary vascular</td>
<td>- Chronic restrictive lung</td>
</tr>
<tr>
<td>congestion</td>
<td>disease</td>
</tr>
<tr>
<td>- Obstructive pulmonary</td>
<td>- Lower lung capacity,</td>
</tr>
<tr>
<td>pattern</td>
<td>exercise intolerance</td>
</tr>
<tr>
<td>- ACS-like</td>
<td>- ACS-like, vascular</td>
</tr>
<tr>
<td>- Stroke, terminal</td>
<td>congestion</td>
</tr>
<tr>
<td>apneas under hypoxia</td>
<td>- Apneas and strokes under</td>
</tr>
<tr>
<td>- Acute respiratory</td>
<td>hypoxia</td>
</tr>
<tr>
<td>failure and lethality</td>
<td></td>
</tr>
</tbody>
</table>

| **57% of SAD pups**      | **52% of SAD survivors**    |
| - slightly lower body    | - milder exercise           |
|   weight                 |   intolerance               |
| - Increased apnea in      | - apneas and strokes        |
|   room air or hypoxia    |   under hypoxia             |
**ADULT SAD ALTERED BREATHING RESPONSE UNDER HYPOXIA**

<table>
<thead>
<tr>
<th>Condition</th>
<th>WT Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room air</td>
<td>Regular sighs followed by brief apnea</td>
</tr>
<tr>
<td>8% O₂ (1 min)</td>
<td>Initial increase in frequency and irregular breathing (blunted $V_E$)</td>
</tr>
<tr>
<td></td>
<td>Prominent period of apneas</td>
</tr>
<tr>
<td>Room air</td>
<td></td>
</tr>
<tr>
<td>8% O₂ (1 min)</td>
<td>SAD$^T$</td>
</tr>
</tbody>
</table>

- WT sustained $V_E$ regular sighs followed by brief apnea
- Adult SAD$^T$ mice have initial increase in frequency and irregular breathing (blunted $V_E$) and prominent period of apneas
SAD MICE ALTERED BREATHING IN NORMOXIA AND HYPOXIA

- Breath/min
- Tidal volume
- Minute ventilation
- Apneas

Plethysmography analysis, approximately half of the adult SAD mice at 7-8 months of age have regular low amplitude, high respiratory frequency – tachypnea

Adult SAD\textsuperscript{T} mice have initial increase in frequency and irregular breathing (blunted \( V_E \)) and prominent period of apneas