Pulmonary Development and Disease in Down Syndrome: a Scientific Journey from the Bed to Bench Site

Csaba Galambos MD, PhD
Department of Pathology and Laboratory Medicine & Pediatric Heart Lung Center, Children’s Hospital Colorado & University of Colorado School of Medicine
Pediatric Pulmonary Vascular Biology & Pathology

Diagnostic Pediatric Pathology (Lung)

Bench Research on Lung Development & Disease
Case study

- 4 month old male with trisomy 21
- born at 36 4/7 weeks gestational age
- birth weight of 3080 grams
- delivered by Cesarean section to a 34 year old G2P1 mother
- multiple respiratory problems since birth including pulmonary hypertension, chronic respiratory failure, and tracheobronchomalacia requiring assisted ventilation
- No cardiac or other organ anomalies
- After a long hospital course without improvement in his cardiorespiratory status, his family decided to withdraw life support and an autopsy permit was granted
Histopathology

Control

Down syndrome

DIMINISHED ALVEOLARIZATION

DEFECTIVE VASCULAR REMODELING

IMPAIRED MICROVASCULAR GROWTH
Cause of Death

*Respiratory*: severe chronic lung disease, pulmonary hypoplasia and pulmonary hypertension related to *Down syndrome*

Babies with DS succumb to respiratory disease without any co-morbid condition
Pulmonary disease and Down syndrome

- DS associated with increased risk for developing respiratory disorders including severe pulmonary hypertension (PHT) and persistent pulmonary hypertension of the newborn (PPHN)
- Prevalence study at Sie Center for Down Syndrome (ongoing):
  - 1,252 children with DS, 27.6% (n = 346) with PHT
  - PPHN incidence: 9.9% (vs 0.1 in general population)
- Children with DS and co-morbidities, such as congenital heart disease or obstructive sleep apnea, are more susceptible for developing accelerated PHT than children without DS
- One of the leading causes of death in people with DS is respiratory related

The genetic and molecular mechanisms responsible for pulmonary hypoplasia and PHT in DS are unknown
Down syndrome associated lung disorders are understudied
Challenges of Down syndrome research

- many genes (300+): *one gene dysfunction*-*one pathway*-*one disease phenotype* paradigm does not apply
- genes are overexpressed
- ~80 clinical phenotypes in various combinations
GENETIC PATHWAYS AND CONFOUNDING FACTORS LEAD TO VARIABLE DS PHENOTYPES

1. 1.5 fold overexpression of trisomic genes ("dosage sensitive genes")

2. up and/or down regulation of disomic genes ("modifiable genes" via transcriptional regulation)

3. Confounding factors (hypoxemia)

80 clinical phenotypes of DS
Are DS-related lung hypoplasia and PHT anti-angiogenic disorders?
Patients with DS have a decreased incidence of pro-angiogenesis related diseases including solid tumors, atherosclerosis, diabetic retinopathy, and vascular anomalies.

Genes for potent anti-angiogenic factors are present on the triplicated chromosome 21 and overexpressed in patients with DS and in DS animal models.
Endostatin (ES), B-amyloid peptide (BAP) and Calcineurin Regulator-1 (RCAN-1/DSCR-1)

- All prominent endogenous anti-angiogenic factors
- All located on Chromosome 21 and all individuals with DS have an extra copy
- Patients with DS have increased serum and/or tissue levels
- They specifically inhibit the proliferation and migration of vascular endothelial cells
- They suppress VEGF-VEGFR2 induced signaling causing marked angiogenesis inhibition

It is unknown whether ES/BAP/RCAN1-related antiangiogenic mechanisms contribute to abnormal lung development and PHT in DS
Overexpression of chromosome 21-related anti-angiogenic factors play a critical role in the development of lung hypoplasia and pulmonary hypertension in infants and children with Down syndrome.
Specific study questions

1. Do lungs in people with DS show impaired alveolar and vascular growth/remodeling?
2. Do lungs of a fetus with Down syndrome overexpress ES, BAP, RCAN-1 and other anti-angiogenic genes (disomic) and show vascular growth impairment?
3. Are angiogenic functions of endothelial cells and progenitors isolated from individuals with DS impaired?
Study question -1

Do lungs in people with DS show impaired alveolar and vascular growth/remodeling?
Study 1- Design

Retrospective Autopsy Review
Children 0-8 years
  With DS or typical with CHD
  Excluded disorders of lung development
Study Population
  Patients with DS (n=13)
  Typical patients age and CHD matched controls (n=4)
Clinical data obtained from autopsy reports
Routine H&E lung histology reviewed
Serial sectioning and 3D image reconstruction to clarify microvascular anatomy
## Study 1-Results

<table>
<thead>
<tr>
<th>Clinical Features</th>
<th>DS (n=13)</th>
<th>non-DS (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital Heart Defect (%)</td>
<td>77</td>
<td>100</td>
</tr>
<tr>
<td>Clinical Diagnosis Pulmonary Hypertension (%)</td>
<td>46</td>
<td>25</td>
</tr>
<tr>
<td><strong>Histologic Features (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary Arterial Hypertensive Remodeling</td>
<td>85</td>
<td>25</td>
</tr>
<tr>
<td>Pulmonary Vein Hypertensive Remodeling</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Prominent Bronchial Vessels</td>
<td>100</td>
<td>25</td>
</tr>
<tr>
<td>Prominent Intrapulmonary Anastomotic Vessels</td>
<td>100</td>
<td>25</td>
</tr>
<tr>
<td><strong>Quantification of Histologic Findings (0-3)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary Arterial Hypertensive Remodeling</td>
<td>1.69</td>
<td>0.5</td>
</tr>
<tr>
<td>Pulmonary Vein Hypertensive Remodeling</td>
<td>0.23</td>
<td>0</td>
</tr>
<tr>
<td>Prominent Bronchial Vessels</td>
<td>2.54</td>
<td>1.5</td>
</tr>
</tbody>
</table>
Study 1-Summary

Infants and Children with DS who died of severe cardiopulmonary disease have:

- Histologic evidence of abnormal lung development
  - Alveolar simplification
- Histologic evidence of abnormal pulmonary vascular development
  - Prominent arterial hypertensive remodeling
  - Prominent bronchial vessels
  - Persistence of double capillary network
  - Prominent intrapulmonary anastomotic vessels

Findings present in those with DS with and without CHD are more prevalent than typical patients who were age and CHD matched controls

Study 1 supports angiogenic hypothesis

D Bush et al. J Peds 2017
Study question-2

Do lungs of a fetus with DS overexpress ES, BAP, RCAN-1 and other anti-angiogenic genes (disomic) and show vascular growth impairment?
Study 2 - Design

Human fetal lung tissue* from
  Down syndrome (n=4)
  Typical controls (n=4)

Individual qPCR (mRNA) and Western Blot Analysis (Protein)
  Endostatin
  B-Amyloid Protein (BAP)
  Regulator of Calcineurin-1 (DSCR1)

Angiogenesis-associated mRNA microarray (84 angiogenic genes)

IHC evaluation for impaired angiogenesis:
  Vessel Density (CD31)
  Vessel Thickness (SMA)

*U. Maryland, Baltimore/NICHD Brain & Tissue Bank for Developmental Disorders
Study 2 - Results

mRNA

- COL18A1: p = 0.0220
- APP: p = 0.0006
- DSCR1: p = 0.226

mRNA

- COL18A1: p = 0.0004

Protein

- ES: p = 0.04
- APP: p = 0.002
- DSCR1: p = 0.05

Control

- Actin
- APP
- Actin
- DSCR1
- Actin

DS
Study 2 - Results
Study 2 - Results

(a) Microvascular Density

Ratio Vascular Area to Parenchyma Area

Control  DS

P = 0.041

P = 0.001

#1  #2

(b) Pulmonary Artery Thickness

Ratio Thickness to Diameter

Control  DS

Control  DS

P = 0.033

#1  #2
Study 2 - Summary

Anti-angiogenic factors are over-expressed in the lungs of fetuses with DS
Chromosome 21-related Genes (trisomic) and Proteins
  *ENDOSTATIN*
  *BAP*
  *RCAN1/DSCR1*
Non Chromosome 21-related Genes (disomic)
  *TIMP3*
  *COL4A3*

Early abnormal vascular growth in the lungs of fetuses with DS
  Reduced vessel density
  Increased vessel wall thickness

*Study 2 supports angiogenic hypothesis*
Study question-3

Are angiogenic functions of endothelial cells and progenitors isolated from individuals with DS impaired?
Study 3-Results (ongoing)

Endothelial cells (HUVECs) and precursors (endothelial colony forming cells) isolated from individuals with DS show impaired angiogenic functions.

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**A**

% Closure Control vs DS HUVEC

<table>
<thead>
<tr>
<th>Control HUVEC</th>
<th>DS HUVEC, p &lt; 0.0001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avg % closure</td>
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% Closure Control vs DS ECFC

<table>
<thead>
<tr>
<th>Control ECFC</th>
<th>DSECFC, p = 0.77</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avg % closure</td>
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**B**

Growth Chart Control vs DS ECFC Cells

![Graph showing growth chart comparison between control and DS ECFC cells across different days (D0, D1, D2, D3).](image)

Legend:
- C-177 ECFC
- C-79 ECFC
- Ts-203 ECFC
- Ts-207 ECFC

Dr. C Baker, PHLC
Disease specific DS mouse models

• to further test our angiogenic hypothesis
• study specific molecular mechanisms
• explore a variety of interventions/treatment options
Mouse models of Down syndrome

No characterization/research of lung disorders has been done in any of DS mouse models

• Mouse orthologues of human Chr21 on 3 separate mouse chromosomes
  • Mmu 10 – ENDOSTATIN (37 genes)
  • Mmu 16 – DSCR1/RCAN1 & BAP* (102 genes)
  • Mmu 17 – NO ANTIANGIOGENIC GENE (17 genes)

Gupta et al, Mamm Genome, 2016
Summary

• Lung disease (PHT/PPHN) is a significant contributor to morbidity and mortality of infants and children with DS
• DS Lung histology is characterized by vascular and alveolar growth abnormalities
• Lung disease in DS is understudied

• Lung pathology of DS is driven by anti-angiogenic mechanisms
• Early intervention with angiogenic stimulators or blockers of anti-angiogenic pathways may prevent lung disease in those with DS
• Angiogenic biomarkers will help predict lung disease in those with DS
Members of Pediatric Heart Lung Center
Angela Minic, Doug Bush, Gregory Seedorf, Blair Dodson, Chris Baker
Dr. Steve Abman—Scientific Director

Members of Dept. of Pathology
Drs. Ann Thor and Mark Lovell

Collaboration
Dr. Charles Hoeffer Lab at University of Colorado Boulder
The Linda Crnic Institute for Down Syndrome—Drs. Fran Hickey, Joaquin Espinosa
Univ. Maryland, Baltimore/NICHD Brain & Tissue Bank for Developmental Disorders

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“Down Syndrome Takes The Breath Away”

“People with DS are gift. By studying their biology can help them and the rest of mankind.”

----Dr Tom Blumenthal, Former Executive Director of LCI for DS

Self-Advocates, Families, & Researcher celebrate World Down Syndrome Day in the CO Capitol together with Governor John W. Hickenlooper (3/22/17)