Fishing for New Therapies for DBA

Camp Sunshine 2017
Elizabeth Macari, PhD
Lab of Leonard Zon, MD
Boston Children’s Hospital
Strengths of studying zebrafish

- Transparent embryo
- Circulating blood in 23 hours
- Transgenic fish
- 100s of progeny / week
- Adult
- Genetic screens
- Gene knockdowns
- Chemical screens
- Conserved blood lineages
Human embryo (28 days)

Zebrafish embryo (19 hours)
Visualizing red blood cells in zebrafish
Two methods to modify zebrafish

Chemical treatment to induce random mutations

Inject genetic material into 1 cell stage
Casper

Transparent, adult zebrafish
Casper lacks pigment of WT fish
Zebrafish blood mutant genes

- **Hematopoietic Stem Cell**
- **Erythroid-progenitor**
- **B cell**
- **T cell**

**Erythroid genes**
- **DBA (rps29)**
- **ret (band 3)**
- **ris (b-spectrin)**
- **sau (alas2)**
- **weh (fpn1)**
- **yqe (urod)**
- **zin (globin)**

**Myeloid genes**
- **bhs**
- **mon (tify)**

**Lymphoid genes**
- **assam**
- **camomile**
- **darjeeling**
- **earl grey (sart 3)**
- **jasmine**

**Additional genes**
- **sis (grx5)**
- **cia (transferrin receptor)**
- **cdy (dmt1)**
- **drc (fch)**
- **frs (mitoferrin)**
- **cha (protein 4.1)**
DBA zebrafish model

Hemoglobin Stain

Wild Type (Healthy Fish)

DBA model
Mutation in rps29
Two methods to modify zebrafish

Chemical treatment to induce random mutations

Inject genetic material into 1 cell stage

This method is useful to add back a gene that is missing.
Human *RPS29* RNA can rescue hemoglobin in *rps29* mutant DBA model

Inject Healthy human (WT) RPS29 RNA
Or RNA from DBA patient

*rsps29/-*

48h

Stain for Hemoglobin

- uninjected
- + WT RNA
- + I31F RNA
- + I50T RNA

*Mirabello L, Macari E et al. Blood 2014*
Chemical screen using DBA zebrafish model

DBA zebrafish model

Chemical libraries
600 compounds

Screen for rescue of hemoglobin

Chemical "hits"
Chemical screen identifies calmodulin inhibitors increase hemoglobin

Stain for Hemoglobin

$rps29^{+/+}$

Vehicle

$rps29^{-/-}$

CaM inhibitors

Trifluoperazine (TFP)
TFP improves anemia in DBA mouse model

**p<0.01

Kavitha Siva and Johan Flygare
TFP increases red blood cells in DBA patient bone marrow samples

RPS19 R94X
Steroid responsive
10 year old patient

BM cells
4 days
TFP 5 days
RBC Differentiation

Analysis for red blood cell markers

Healthy bone marrow
DBA patient bone marrow

GLYA

CD71

89
42.1
47.2

20
3.34
17

50
7.1
43

Sergei Doulatov and Linda Vo
Patient derived induced pluripotent stem (iPS) cells

Too few patient blood cells → Skin cells → Patient fibroblasts: RPS19 RPL5 → Respecify → Patient-derived iPS cells → Blood cells

Disease modeling and drug screens
DBA iPS cells show impaired ability to make red blood cells

DBA patient skin cells → iPS cells → RBC Differentiation → Analysis for red blood cell markers and hemoglobin

Control | RPS19+/− | RPL5+/− | Control heme | RPL5+/− heme

GLYA

CD71

33 | 11 | 10
Chemical screen using DBA iPS cells

DBA patient derived iPS cells

Chemical libraries
1200 compounds

Screen for chemicals that give more red blood cells

Chemical “hits” SMER 28
SMER28 increases number of red blood cells

- iPS cells
- RBC Differentiation
- SMER28
- Analysis for red blood cell markers

Control

RPL5+/− DBA Cells

RPL5+/− DBA Cells +SMER28

Healthy DBA RPS19 mutant cells

GLYA

CD71

89

54

76.5

42.1

6.16

47.2

47.9

26

50.5

1.0 µM SMER28
SMER28 increases red blood cells in DBA patient samples

BM cells 4 days

RBC Differentiation

SMER28

Analysis for red blood cell markers

DBA Patient 1
RPS19

DBA Patient 2
RPS29

untreated

SMER28 1.0 µM

GLYA

CD71

untreated

SMER28 1.0 µM

20 3.34 17.1

55 10.7 44.2

15 0.75 14.1

41 2.4 38.3
SMER28 improves hemoglobin in DBA zebrafish model

Healthy (WT)  
DBA  
DBA 1µM SMER28
Chemical screens to discover potential therapies to treat DBA

DBA zebrafish model

DBA patient derived iPS cells

Clinical trial starting in adults

TFP

SMER28

Further testing and better derivatives needed
Acknowledgements

Zon Lab

Len Zon
Alison Taylor
Emma Stillman
Julia Huber
Kate McGrath
Serine Avagyan
Julien Ablain
Julie Perlin
Christie Ciarlo
Jess Humphries
Alessia Giannozzi
Entire Zon Lab!

Kara Maloney
Chris Lawrence
Bruce Barut
Hannah diCicco

Daley Lab
Sergei Doulatov
Linda Vo

Ebert Lab
Dave Raiser
Anu Narla

BCH FACS Core
Ronald Mathieu
Mahnaz Paktinat

Lund University
Johan Flygare
Kavitha Siva

Northwell Health
Jeff Lipton
Adrianna Vlachos

NCI
Blanche Alter
Lisa Mirabello
Sharon Savage

DBA patients and their families

Funding
NHLBI F32 and NHBLI U01

The Taub Foundation
Grants Program for MDS Research

A PROGRAM OF
THE HENRY AND MARILYN
TAUB FOUNDATION