AWARD NUMBER: W81XWH-20-1-0812

TITLE: Microenvironmental Effect on Red Cell Generation in MDS

PRINCIPAL INVESTIGATOR: Zack Wang

CONTRACTING ORGANIZATION: Johns Hopkins University, Baltimore, MD

REPORT DATE: September 2021

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
September 2021	Annual	15Aug2020-14Aug2021

4. TITLE AND SUBTITLE

Microenvironmental Effect on Red Cell Generation in MDS

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-20-1-0812

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Zack Wang, PhD

E-Mail: zwang51@jhmi.edu

5d. PROJECT NUMBER
0011491427-0001

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Johns Hopkins University, The
3400 North Charles Street
Baltimore, MD 21218-2608

8. PERFORMING ORGANIZATION REPORT NUMBER
001

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT: Anemia is the predominant clinical manifestation of MDS, especially at early stages. Erythropoiesis is a tightly regulated and complex process that requires dynamic interactions among hematopoietic stem and progenitor cells (HSPCs) with bone marrow (BM) microenvironments (also called cellular niches) to allow lineage commitment of erythroid progenitors (erythroblasts), accumulation of erythroblast, and terminal differentiation of erythroblasts to give rise to enucleated erythrocytes. The molecular pathogenesis of dysplastic erythropoiesis in MDS is largely unknown partially due to a lack of a system to study erythropoiesis in a stage-specific fashion. The goal of this project is to investigate how the intrinsic defects of erythropoiesis interact with extrinsic effects of microenvironments to result in impaired erythropoiesis in MDS. To understand the diverse mechanisms of erythroid dysplasia of MDS, we would employ our established in vitro sequential niche system to investigate the microenvironment effect on erythropoiesis and dysplastic erythropoiesis in MDS. We anticipate that diverse cellular niche cues play distinct roles in promoting the development of erythroblasts and erythroid maturation, and MDS-HSPCs either are unable to reliably communicate with niche cues or exert a forward influence on microenvironments to interfere with cellular niche function. Our designed niche system is relatively simple, based on two major stages of erythropoiesis: 1) endothelial cell (EC) niche for HSPC expansion and erythroid lineage commitment; and 2) BM stromal cell (BMSC) niche for erythroblast proliferation and maturation. In this project, we will use this niche system 1). To characterize dysplastic erythroid commitment of Low Risk-Myelodysplastic Syndromes (LR-MDS) by endothelial cell (EC) niche; 2). To examine the maturation capability of early erythroblasts derived from Myelodysplastic Syndromes (MDS) on bone marrow stromal cell (BMSC) niches.

15. SUBJECT TERMS None listed.					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified	Unclassified	7	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

1. Introduction

Myelodysplastic syndromes (MDS) are heterogeneous diseases with diverse clonal hematopoietic disorders of hematopoietic stem cells (HSCs) and/or lineage committed progenitor cells that result in ineffective hematopoiesis. In this project, we will focus on the non-inherited refractory anemia subtype of MDS that has a low risk (LR) in transforming to leukemia. Anemia is the predominant clinical manifestation of LR-MDS patients, and the molecular pathogenesis of dysplastic erythropoiesis is largely unknown partially due to a lack of experimental system that allow stage-specific interrogation of erythropoiesis, such as erythroid lineage commitment from HSCs, erythroblast proliferation and maturation, and dysfunction of microenvironment that leads to dysplastic erythropoiesis. Erythropoiesis in adult undergo multiple stages: 1) differentiation of HSCs to common myeloid progenitors (CMPs); 2) commitment of CMPs to lineage-restricted early erythroid progenitors (proerythroblasts or pronormoblasts); 3) maturation of pronormoblast to give rise to basophilic erythroblasts, then polychromatic erythroblasts, and then orthochromatic erythroblasts; and 4) terminal differentiation of erythroblasts to generate mature enucleated erythrocytes (reticulocyte) after expelling the nucleus. In adult, erythropoiesis occurs the BM, where exist a rich cellular microenvironment. The failure of erythropoiesis in MDS might results from defective HSCs and erythroid progenitors, or from impaired bone marrow microenvironments. The role of the microenvironment in MDS manifestation and progress is controversial due to the heterogeneous nature of MDS, the complexity of erythropoiesis, and inconsistency in study systems. Because of a lack of appropriate experimental systems to delineating the defective stages at which dyserythropoiesis occurs, it is also largely unknown how dysfunction of a microenvironment contributes to dyserythropoiesis of MDS. The goal of this project is to investigate intrinsic defects of MDS-HSPCs and extrinsic cellular niche defects in microenvironments that impair erythropoiesis.

2. Keywords

MDS- myelodysplastic syndromes
BMF- bone marrow failure
HSPCs- hematopoietic stem and progenitor cells
HSCs- hematopoietic stem cells
RBC- Red blood cell
BMSCs- bone marrow stromal cells
EPO- erythropoietin
hPSCs- human pluripotent stem cells
ECs- endothelial cells
HE- hemogenic endothelial
OP9- mouse bone marrow stromal cells
DLL1- Notch Delta ligand Dll1

3. Accomplishments

What were the major goals of the project?

The major goals of the project are 1). To characterize dysplastic erythroid commitment by endothelial cell (EC) niche; and 2). To examine the maturation capability of early erythroblasts derived from Myelodysplastic Syndromes (MDS) on bone marrow stromal cell (BMSC) niches.

What was accomplished under these goals?

Because of COVID-19 pandemic, our ability of accessing patient sample has been limited during this reporting period. Our activities focused on erythropoiesis of hPSCs in vitro. We found that Notch signaling plays divergent roles in respective niche functions at different stages during erythroid differentiation and maturation. Notch signaling plays an essential role in regulating endothelial niche functions for promoting the development of HSPCs. The role of Notch signaling in regulating erythropoiesis is unclear and controversial. Our preliminary study demonstrated that ECs elicited niche function on erythroblasts through Notch signaling, leading to upregulation of Notch downstream targets HES1, HES5 and HEY1. Notch γ-secretase inhibitor (GSI) effectively blocked this Notch effect. Although the presence of GSI during EC co-culture affected neither frequency nor number of CD235a+CD41- erythroblasts, GSI rendered the EC-primed erythroblasts unable to generate CD235+H33342- enucleated erythrocytes in the following OP9 BMSCs co-culture. We also investigated the Notch effect on erythroid maturation by replacing OP9 BMSC with OP9-DLL1 cells that ectopically express Notch ligand Delta-like 1 (DLL1). OP9-DLL1 significantly increased the gene expression of Notch downstream targets, HES1, HES5, and HEY1 in the erythroid cells, indicating an elevated Notch activation, which coincided with decreased expression of hemoglobin and less enucleated erythrocytes. Taking together, these results indicated that the Notch signaling is a key mediator in niche function during

erythropoiesis, and our cellular niche system is adequate for in vitro analysis of cellular and molecular mechanisms that affect erythropoiesis of HSPCs and MDS-HSPCs.

What opportunities for training and professional development has the project provided?
Nothing to Report.

How were the results disseminated to communities of interest?
Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?
We will further investigate how hematopoietic cells will differentiate effectively into lineage committed CD235a+CD71+ erythroblasts on the provided EC niche. We expect to observe a failed erythroblast development indicated by an absence or reduced production of early erythroblasts. Further study of cells from MDS patients will be used to determine whether our finding is consistent in patient-derived cells from LR-MDS.

4. Impact

What was the impact on the development of the principal discipline(s) of the project?
Our investigation will provide new insights into regulation of erythropoiesis at normal and diseased context, provide a new platform for further investigation of the cellular and molecular mechanisms of erythroid differentiation and enucleation in MDS, and shed light on the development of alternative treatment aimed at reducing the frequency of RBC transfusion and improving the quality of life of MDS patients.

What was the impact on other disciplines?
This project will provide insights into the role of microenvironment in regulating blood cell generation from hPSCs.

What was the impact on technology transfer?
Nothing to Report.

What was the impact on society beyond science and technology?
Nothing to Report.

5. Changes/problems

Changes in approach and reasons for change
Because of COVID-19 pandemic, our ability of accessing patient sample has been limited during this reporting period. Therefore, our activities have been focused on microenvironmental effect on blood cell generation from hPSCs in vitro.

Actual or anticipated problems or delays and actions or plans to resolve them
In a case of continuing patient sample limitation, we will dissect the molecular and cellular mechanisms of niche in blood cell generation in vitro.

Changes that had a significant impact on expenditures
Based on the study of microenvironmental effect on blood cell generation in vitro during this reporting period, we expect that Notch signaling positively regulates arterial endothelial niche function that promotes the development of HSPCs.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to Report.

6. Products

Nothing to Report.

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?

Name	Zack Wang	Amy DeZern	Yuting Huang

Project Role	PI	Co-PI	Post-doc fellow
Nearest person month worked:	6	1	6
Contribution to Project:	experimental direction and data interpretation	discuss the progress and MDS BM samples	experiments and data analysis

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to Report.

What other organizations were involved as partners?
Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS
Nothing to Report