PKM and *PKR* Expression During Hematopoiesis and Erythropoiesis

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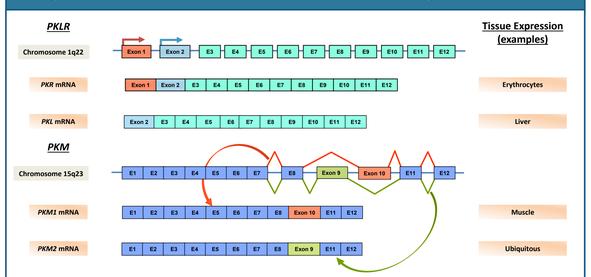
OBJECTIVE

To understand relative expression and potential roles of PK isoforms during normal hematopoiesis and erythropoiesis

BACKGROUND

- Pyruvate kinase (PK) is a key enzyme in the glycolytic pathway and thus essential for cell metabolism
- PK is needed to produce adenosine triphosphate (ATP), which is essential for meeting the energy demands of erythrocytes[1]
- 4 tissue-specific PK isoforms are encoded by 2 genes (**Figure 1**)[2]
  - ***PKLR*** encodes the PKL and PKR isoforms through tissue-specific promoters
  - ***PKM*** encodes the PKM1 and PKM2 isoforms through alternative splicing[2]
  - mRNA expression of PK-associated genes varies throughout the stages of normal hematopoiesis and erythropoiesis[3]

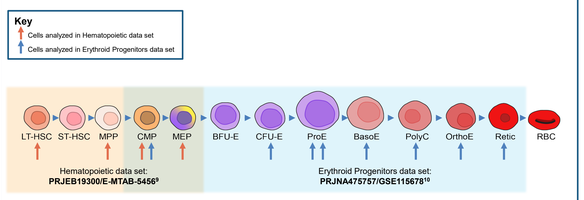
Figure 1. Four PK Isoforms Are Encoded by 2 Genes[2,4-6]



E, exon; PK, pyruvate kinase; PKL, liver-type pyruvate kinase; PKM1/2, muscle-type pyruvate kinase 1/2; PKR, red blood cell-type pyruvate kinase.

- A lack of PK dysregulates hematopoiesis and erythropoiesis and can lead to such diseases as thalassemia, myelodysplastic syndrome-associated anemia, sickle cell disease, and PK deficiency[7-10]
- To better understand how to treat these diseases, it is helpful to understand PKM and PKR expression during normal hematopoiesis and erythropoiesis
- In this study, transcriptomes from hematopoietic and erythroid progenitors were evaluated, and mRNA levels of *PKL, PKR, PKM1,* and *PKM2* were measured at different stages of hematopoiesis and erythropoiesis (**Figure 2**)

Figure 2. Hematopoiesis, Erythropoiesis, and Human Data Sets[11,12]



BasoE, basophilic erythroblast; BFU-E, burst-forming unit–erythroid; CFU-E, colony unit–erythroid; CMP, common myeloid progenitor; LT-HSC, long-term hematopoietic stem cell; MEP, megakaryocyte erythroid progenitor; MPP, multipotent progenitor; OrthoE, orthochromatic erythroblast; PolyC, polychromatic erythroblast; ProE, proerythroblast; RBC, red blood cell; Retic, reticulocyte; ST-HSC, short-term hematopoietic stem cell.

METHODS

- Two RNA-sequencing (RNA-seq) data sets were obtained from public functional genomics data repositories
  - **Published method for generating ex vivo data set PRJEB19300/E-MTAB-5456[11]:**
    - Early hematopoietic progenitors (CD34$^+$ cells depleted for those expressing lineage commitment markers [Lin$^-$]) were isolated from umbilical cord blood (obtained from healthy donors)
    - Subsets were isolated, evaluated by flow cytometry, and verified by staining (**Table 1**)
    - 100 cells were directly sorted into lysis buffer before further RNA-seq assay processing
    - 4 replicate transcriptomes were run per cell type

Table 1. Human Hematopoiesis Ex Vivo Data Set[11]

Cell Type	Cell Surface Marker Expression					
	CD34	CD90	CD38	CD123	CD45RA	CD10
HSC	+	+	–	–	–	–
MPP	+	–	–	–	–	–
CMP	+	–	+	+	–	–
MEP	+	–	+	–	–	–

CMP, common myeloid progenitor; HSC, hematopoietic stem cell; MEP, megakaryocyte erythroid progenitor; MPP, multipotent progenitor.

  - **Published method for generating in vitro data set PRJNA475757/GSE115678[12]:**
    - CD34$^+$ erythroblast populations were differentiated from healthy adult human donors using a 3-phase erythroid differentiation protocol[13]
      - Produced in 3 or 4 replicates using cultured cells from 2 or 3 healthy adult human donors
    - Subpopulations enriched for 8 different stages of maturation were isolated using gated flow cytometry-activated cell sorting (FACS; **Table 2**)
      - Erythroid surface markers used: CD71, CD235a, CD49d, and Band 3 (encoded by the *SLC4A1* gene)
    - Each enriched subpopulation was processed using RNA-seq[14]
      - 28 paired-end RNA-seq libraries were produced and sequenced

Table 2. Human Erythropoiesis In Vitro Data Set[12]

Cell Type	Cell Surface Marker Expression			
	CD71	CD235a	CD49d	BAND3
CMP	Low	Low	None	None
CFU-E	High	Low	None	None
ProE1	High	Medium	None	None
ProE2	High	High	None	None
BasoE	None	None	High	Low
PolyC	None	None	High	Medium
OrthoE	None	None	Medium	High
Retic	None	None	Low	High

BasoE, basophilic erythroblast; CFU-E, colony-forming unit–erythroid; CMP, common myeloid progenitors; OrthoE, orthochromatic erythroblast; PolyC, polychromatic erythroblast; ProE, proerythroblast; Retic, reticulocyte.

  - **Processing of raw sequencing data**
    - Raw sequencing data from 2 public repositories were processed by FastQC and Trimmomatic for quality control and adapter trimming[15,16]
    - The processed data were mapped to the transcriptome using Spliced Transcripts Alignment to a Reference (STAR)[17]
    - Transcript quantification values were then calculated using RNA-Seq by Expectation-Maximization (RSEM)[18]
    - RSEM-normalized data were pre-processed to provide a transcripts-per-million (TPM) matrix
    - *PKL*, *PKR*, *PKM1*, and *PKM2* mRNA expression data were visualized using RStudio[19]

RESULTS

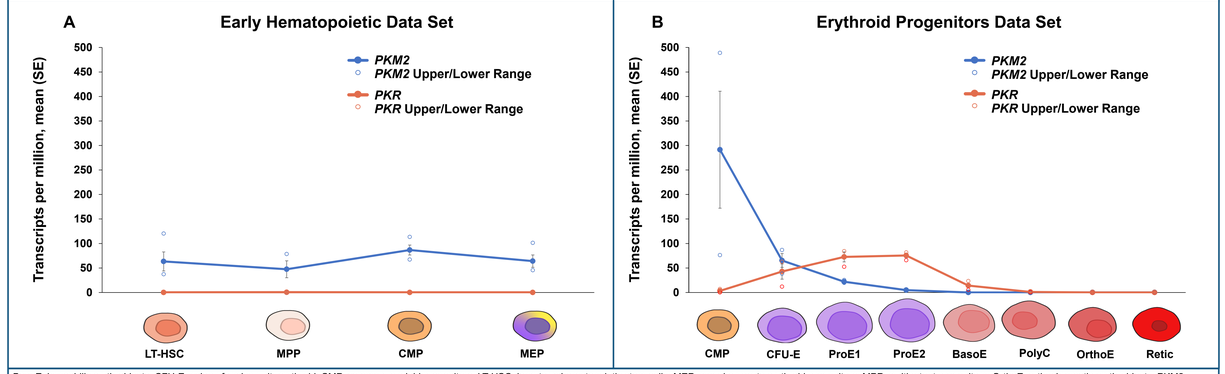
- Early hematopoietic data set
  - *PKR*, *PKL*, and *PKM1* mRNA expression was low-to-undetectable in all cell types (**Table 3**)
  - *PKM2* mRNA was present in early hematopoiesis and was expressed in LT-HSC, MPP, CMP, and MEP cell types (**Figure 3A**)
- Erythroid progenitors data set
  - *PKM1* and *PKL* mRNA expression was low-to-undetectable in all cell types (**Table 3**)
  - *PKM2* and *PKR* mRNAs were both expressed in early erythropoiesis
    - *PKM2* mRNA was dominant in CMP and CFU-E cell types (**Figure 3B**)
    - *PKR* mRNA was dominant in ProE1, ProE2, and BasoE cell types (**Figure 3B**)
  - Both *PKM2* and *PKR* mRNA expression decreased upon maturation (**Figure 3B**)

Table 3. Expression of *PKLR* and *PKM* Genes in Hematopoiesis and Erythropoiesis[a]

	PKR			PKL			PKM1			PKM2		
Early Hematopoietic Data Set	min	mean	max	min	mean	max	min	mean	max	min	mean	max
LT-HSC	0	0.02	0.03	0	0	0	0	0.18	0.71	37.31	63.39	120.30
MPP	0	0.43	1.69	0	0.01	0.02	0	0.37	1.47	0	47.38	78.52
CMP	0	0.01	0.04	0	0	0	0	0.09	0.21	67.31	86.70	113.35
MEP	0	0.09	0.26	0	0.01	0.04	0	0.79	2.05	45.47	64.03	101.33
Erythroid Progenitors Data Set												
CMP	0.15	3.36	7.14	0	0.10	0.29	0.8	1.02	1.19	76.3	291.36	488.96
CFU-E	11.94	42.76	62.62	0	0.86	2.58	0	0.23	0.38	38.46	65.34	86.83
ProE1	52.47	72.75	84.36	0	0.84	1.73	0	0	0	19.71	22.05	25.54
ProE2	65.79	75.51	81.80	0	1.56	3.81	0	0	0	3.96	4.84	6.33
BasoE	5.44	14.11	23.45	0	0.28	0.84	0	0	0	0	0.07	0.26
PolyC	0.21	1.16	2.13	0	0.03	0.11	0	0	0	0	0.07	0.18
OrthoE	0	0.09	0.20	0	0.02	0.03	0	0	0	0.13	0.26	0.39
OrthoE and Retic	0	0.02	0.08	0	0.01	0.02	0	0	0	0.11	0.27	0.47

[a]Data are presented as mean (minimum, maximum) transcripts per million.
BasoE, basophilic erythroblasts; CFU-E, colony-forming unit – erythroid; CMP, common myeloid progenitors; LT-HSC, long-term hematopoietic stem cells; MEP, megakaryocyte erythroid progenitors; MPP, multipotent progenitors; OrthoE, orthochromatic erythroblasts; PolyC, polychromatic erythroblasts; ProE, proerythroblasts; Retic, reticulocytes.

Figure 3. *PKM2* and *PKR* Expression During Hematopoiesis and Erythropoiesis



BasoE, basophilic erythroblasts; CFU-E, colony-forming unit–erythroid; CMP, common myeloid progenitors; LT-HSC, long-term hematopoietic stem cells; MEP, megakaryocyte erythroid progenitors; MPP, multipotent progenitors; OrthoE, orthochromatic erythroblasts; PKM2, muscle-type pyruvate kinase 2; PKR, red blood cell-type pyruvate kinase; PolyC, polychromatic erythroblasts; ProE, proerythroblasts; Retic, reticulocytes.

CONCLUSIONS

- ***PKM1* and *PKL* transcripts were present at low-to-undetectable levels in both hematopoietic and erythroid progenitors**
- ***PKM2* transcripts were present at early stages of hematopoiesis; *PKR* transcripts were present at low-to-undetectable levels**
- **Importantly, *PKM2* and *PKR* transcripts were both expressed in early erythropoiesis and then decreased upon maturation**
- **These data describe levels of mRNA expression; protein levels were not assessed**
- **Similar studies of transcriptomes derived from patients with diseases that feature dysregulated hematopoiesis and ineffective erythropoiesis may inform the future design of effective PK-targeted pharmacotherapeutic approaches**

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DISCLOSURES

All authors are employees and equity holders in Agios Pharmaceuticals, Inc.

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