



# Generation and examination of immortalized human erythroid progenitor cell line from hematopoietic stem cells derived from $\beta^0$ -thalassemia (codons 41/42) with hemoglobin E disease

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## Introduction

- Codons 41/42 (-TTCT) or the 4 base-pair deletion is one of the most common  $\beta^0$ -thalassemia mutations in Thailand, causing the absence of  $\beta$ -globin production.
- Coinheritance of  $\beta^0$ -thalassemia (codons 41/42) and hemoglobin E ( $\beta^0$ -thalassemia/HbE) accounts for one of the main genotypes of severe thalassemia cases globally, with high prevalence in Southeast Asia, especially Thailand, where an estimated 3,000 children are born annually, and 100,000 patients are living with the condition.
- The study of deep molecular pathology and drug discovery in this common  $\beta^0$ -thalassemia/HbE disease is notably restricted by the shortage of patient-derived materials and the unavailability of appropriate cell line models.
- In this study, we generated an immortalized erythroid cell line from peripheral blood CD34<sup>+</sup> hematopoietic stem cells.

## Methodology

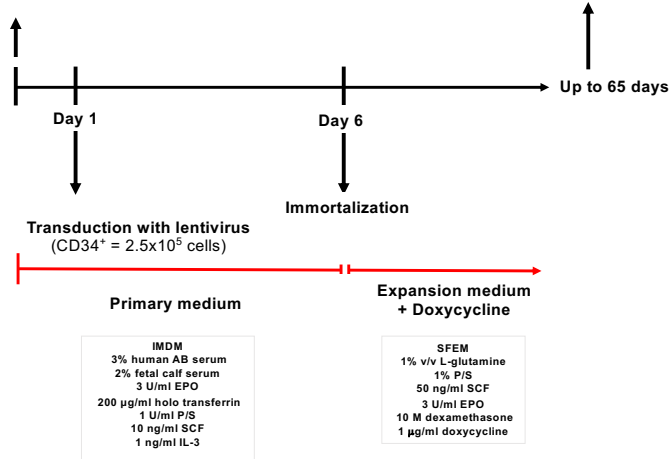
### Experimental conditions

- Control (no lentivirus)
- Immortalized (with lentivirus)

### Characterization of immortalized erythroid progenitor cells

- Cell proliferation
- Cell viability
- Maturation
- Hemoglobin production

### CD34<sup>+</sup> cells isolation



### Subject

- Peripheral blood CD34<sup>+</sup> hematopoietic stem cells from a Thai woman affected by compound heterozygous  $\beta^0$ -thalassemia [codons 41/42 (-TTCT)] and HbE [codon 26 (G>A)]

### Methods

- Lentivirus carrying Tet-inducible HPV16 E6/E7 gene expression was produced in HEK293T cells
- CD34<sup>+</sup> hematopoietic stem/progenitor cells were isolated from peripheral blood of the patient and were transduced with the produced virus carrying HPV16 E6/E7 genes.
- Doxycycline was supplemented in the culture system to induce the expression of HPV16 E6/E7 genes.
- Specific biological properties and characteristics of the produced immortalized erythroid cell line were investigated by identifying the immortalization of erythroid progenitor cells through sub-culturing for more than 60 days

## Conclusion

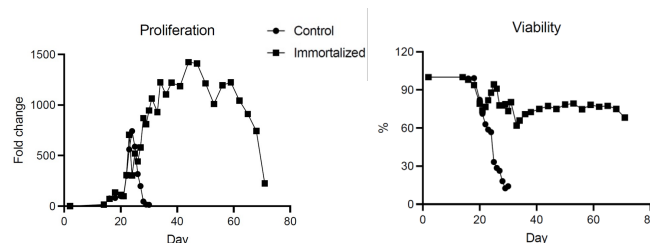
- This study generated the first human cellular model for the common  $\beta$ -thalassemia disease genotype, compound heterozygous  $\beta^0$ -thalassemia [codons 41/42 (-TTCT)] with HbE [codon 26 (G>A)], providing a potentially sustainable platform for studying disease mechanisms and therapeutic strategies.

## Acknowledgement

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## Results and discussion

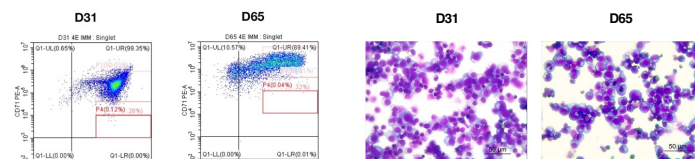
### 1. Immortalized cells can maintain cell proliferation and viability up to over 60 days in *in vitro* culture



- To verify the indefinite proliferation and long-term culture potential of immortalized cells, we assessed the proliferation and viability of CD34<sup>+</sup> cells under two experimental conditions during sub-culture.

- The study demonstrated that cells transfected with a lentivirus containing the HPV16 E6/E7 gene exhibited the ability to proliferate for up to 60 days, maintaining a cell viability of approximately 60-70% after 30 days in culture. In contrast, control cells, which were not transfected with the virus, failed to proliferate beyond 30 days and experienced a decline in viability, ultimately dying by day 30. These findings indicate that cells transfected with the HPV16 E6/E7 gene achieved full immortalization, allowing for long-term proliferation and viability compared to the control cells.

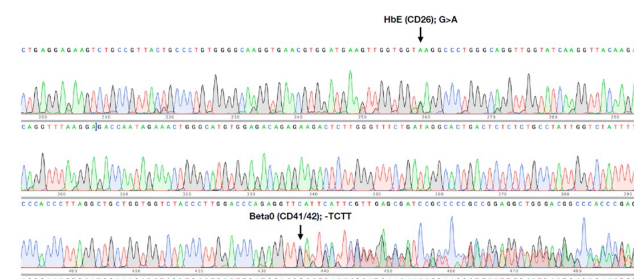
### 2. Majority of the immortalized cells are in basophilic erythroblast stage in culture



- To investigate the maturation of erythroid progenitor cells in culture, we collected cells on days 31 and 65 and analyzed their erythroid stage by measuring the expression levels of two erythroid-specific surface markers, CD71 and GPA, using flow cytometry. The results were then confirmed with Wright-Giemsa staining.

- The results demonstrated that the cells highly expressed CD71 and GPA, corresponding to basophilic erythroblasts in Wright-Giemsa staining. This finding suggests that the immortalized cells could not further proliferate beyond day 60 may be due to the pathophysiological condition of  $\beta^0$ -thalassemia [codons 41/42 (-TTCT)] with HbE [codon 26 (G>A)] disease, characterized by imbalanced globin synthesis at the basophilic erythroblast stage. However, these immortalized cells can recapitulate the ineffective erythropoiesis of  $\beta^0$ -thalassemia [codons 41/42 (-TTCT)] with HbE [codon 26 (G>A)] disease under *in vitro* conditions.

### 3. No change in genetic mutation of immortalized erythroid progenitor cells of $\beta^0$ -thalassemia/HbE



- To investigate the genetic mutation of immortalized erythroid progenitor cells, we collected the cells, amplified the HBB gene, and verified the mutations using Sanger sequencing.

- The results revealed the presence of the codons 41/42 (-TTCT) and codon 26 (G>A) mutations in the immortalized erythroid progenitor cells cultured for 65 days, consistent with the genotype from peripheral blood cells. This indicates that there was no change in the genetic mutations of the erythroid progenitor cells before and after immortalization with the HPV16 E6/E7 gene. Therefore, these cells can be served as a model for  $\beta^0$ -thalassemia [codons 41/42 (-TTCT)] with HbE [codon 26 (G>A)].