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# Transdifferentiation of erythroblasts to megakaryocytes using FLI1 and ERG transcription factors

## Research of the month
## June 2015
## Multidisciplinary research

Impact factor = 4.984

# Transdifferentiation of erythroblasts to megakaryocytes using FLI1 and ERG transcription factors

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## Summary

Platelet transfusion has been widely used to prevent and treat life-threatening thrombocytopenia; however, preparation of a unit of concentrated platelet for transfusion requires at least 4–6 units of whole blood. At present, a platelet unit from a single donor can be prepared using apheresis, but lack of donors is still a major problem. Several approaches to produce platelets from other sources, such as haematopoietic stem cells and pluripotent stem cells, have been attempted but the system is extremely complicated, time-consuming and expensive. We now report a novel and simpler technology to obtain platelets using transdifferentiation of human bone marrow erythroblasts to megakaryocytes with overexpression of the *FLI1* and *ERG* genes. The obtained transdifferentiated erythroblasts (both from CD71$^{+}$ and GPA$^{+}$ erythroblast subpopulations) exhibit typical features of megakaryocytes including morphology, expression of specific genes (*cMPL* and *TUBB1*) and a marker protein (CD41). They also have the ability to generate megakaryocytic CFU in culture and produce functional platelets, which aggregate with normal human platelets to form a normal-looking clot. Overexpression of *FLI1* and *ERG* genes is sufficient to transdifferentiate erythroblasts to megakaryocytes that can produce functional platelets.

## Keywords

Transdifferentiation, erythroblast, megakaryocyte, platelet, transcription factors

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# Transdifferentiation of erythroblasts to megakaryocytes using FLI1 and ERG transcription factors

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# Transdifferentiation of erythroblasts to megakaryocytes using FLI1 and ERG transcription factors

A

RSV | 5'LTR | Ψ | RRE | $P_{UbC}$ | FLI1 | $P_{SV40}$ | 3'LTR

B

RSV | 5'LTR | Ψ | RRE | $P_{UbC}$ | ERG | $P_{SV40}$ | 3'LTR

C

BM-derived
CD71+ or GPA+

Lentiviral transduction

4 h

Transduced erythroblasts

CD71⁺ GPA⁺

Lentiviral production
(*FLI-1, ERG* gene)

293 FT cell plating

Protocol 1: Liquid-based system

Generation of iMKs

Expansion of iMKs

Platelet production
(OP9 co-culturing)

Protocol 2: Semi-solid collagen-based system

Colony forming unit-megakaryocyte (CFU-Mk) production

Day of culture

-3 -2 0 1 7 11

**Figure 1:** Plasmid vectors and culture protocols used in this study. A) Diagram describing FLI1/pLenti6/V5-DEST vector; B) Diagram describing ERG/pLenti6/V5-DEST vector; Diagram describes culture protocols used in this study.

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**Table 1:** List of primers for qRT-PCR analysis.

| Gene | Forward primer | Reverse primer |
|---|---|---|
| *FLI1* | CCCACCAGCAGAAGGTGAAC | ATGCGGCTCCAAAGAAGCT |
| *ERG* | AGACCTGGCGGAGGCTTTT | ATGTTCTCCGATAGAGTTTGTGGC |
| *EPOR* | ACCCCAAGTTCGAGAGCAA | GTAGGCAGCGAACACCAGA |
| *CD71R* | GTCGCTGGTCAGTTCGTGATT | GCATTCCCGAAATCTGTTGT |
| *c-MPL* | GCTAGCTCCCAAGGCTTCTT | GTGGTCACCTCCACAAGGAT |
| *TUBB1* | GGGAGATGATTGGTGAGGAA | CTGTCGGGTTGAAAGAGAGC |

*FLI1* = Friend leukaemia integration factor 1, *ERG* = E-twenty six–related gene, *EPOR* = Erythropoietin receptor, *CD71R* = Transferrin receptor, *c-MPL* =Thrombopoietin receptor, *TUBB1* = β1-Tubulin.

# Transdifferentiation of erythroblasts to megakaryocytes using FLI1 and ERG transcription factors

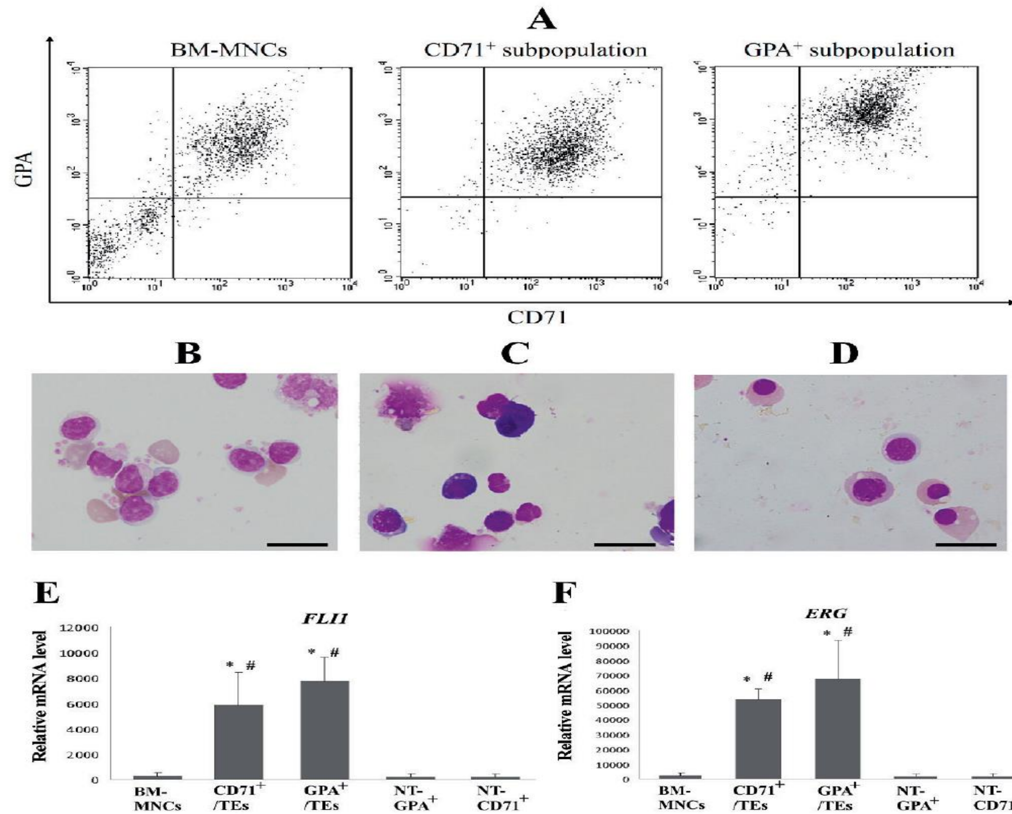
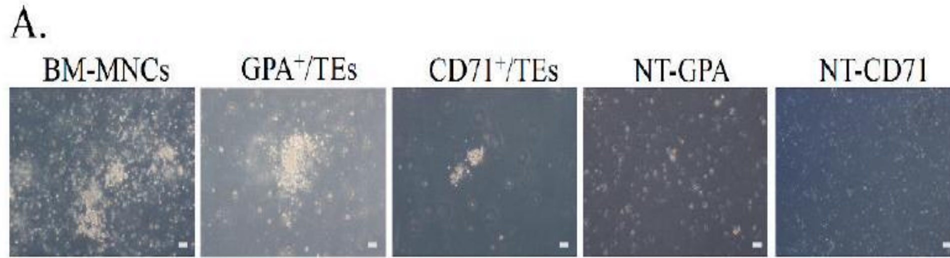



**Figure 2:** Characteristics of $CD71^+$ and $GPA^+$ subpopulations and expression levels of FLI1 and ERG genes in $CD71^+$/TEs and $GPA^+$/TEs. A) Representative dot plots show $CD71^+$ and $GPA^+$ cells in BM-MNCs, $CD71^+$ and $GPA^+$ subpopulations as determined by flow cytometry. B-D) Representative micrographs show morphology of freshly isolated BM-MNCs (B), freshly isolated $CD71^+$ subpopulation (C) and freshly isolated $GPA^+$ subpopulation (D) as determined by Wright's staining. The images were captured by Nikon Eclipse Ti microscopy (Melville, NY, USA) using NIS Elements D4.10.00 software. Scale bar: 20 μm. E-F) Graphs show relative mRNA level of exogenous FLI1 (E) and ERG gene (F) of BM-MNCs, $CD71^+$/TEs and $GPA^+$/TEs at day 4 post-transduction. The freshly isolated $CD71^+$ and $GPA^+$ subpopulations were transduced with FLI1/pLenti6/V5-DEST and ERG/pLenti6/V5-DEST vectors using lentiviral transduction system. At day 4 post-transduction, the expression levels of exogenous FLI1 and ERG genes in transduced erythroblasts ($CD71^+$/TEs and $GPA^+$/TEs) were determined by qRT-PCR. Expression levels of FLI1 and ERG genes of the non-transduced $GPA^+$ (NT-$GPA^+$) and the nontransduced $CD71^+$ (NT-$CD71^+$) served as reference controls. Data were presented as mean ± SD of three independent experiments. The Mann-Whitney U test was used to assess the significance of differences between the observed data. *$P \leq 0.05$ vs BM-MNCs, #$P \leq 0.05$ vs non-transduced erythroblasts.

# Transdifferentiation of erythroblasts to megakaryocytes using FLI1 and ERG transcription factors



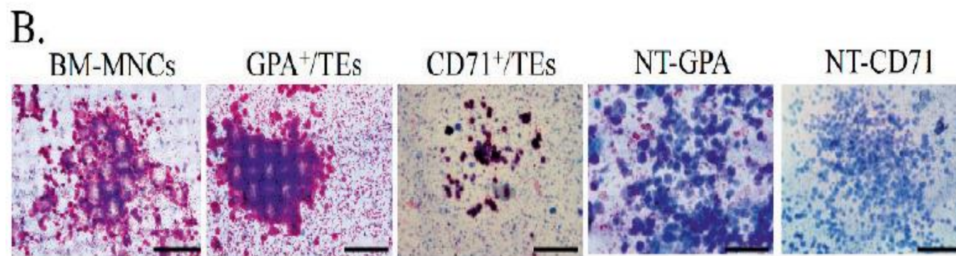




**Figure 3:** Ability of $CD71^+$/TEs and $GPA^+$ /TEs to generate CFU-MK. A) Representative micrographs show morphology of CFU-MK derived from the $CD71^+$/TEs, the $GPA^+$/TEs and BM-MNCs after being cultured on collagenbased megakaryocyte inducing culture conditions for 11 days. NT-$GPA^+$ and NT-$CD71^+$ cultured under the same conditions served as controls. Scale bar: 100 µm. B) Representative micrographs show characteristics of CFU-MK derived from $CD71^+$/TEs, $GPA^+$/TEs and BM-MNCs at culture day 11 after staining with antibody against human glycoprotein IIb/IIIa (GPIIb/IIIa) antibody (red). Cell nuclei were visualised by Evan's blue staining (blue). Scale bar: 50 µm. The images were captured by Nikon Eclipse Ti microscopy (Melville, NY, USA) using NIS Elements D4.10.00 software.

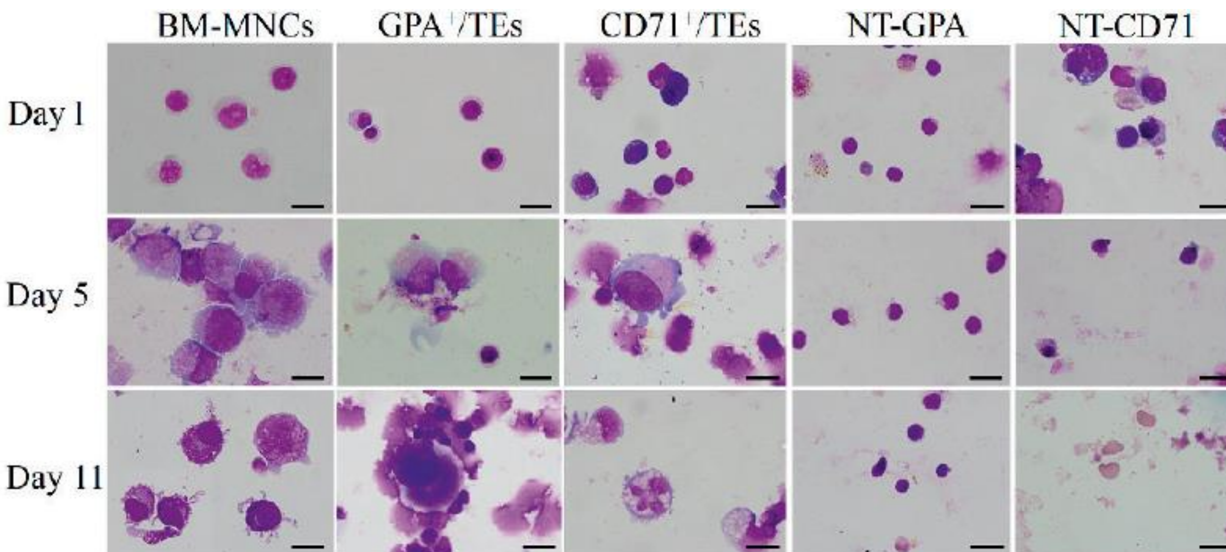



**Figure 4:** Morphology of $CD71^+$/TEs and $GPA^+$/TEs. $CD71^+$/TEs and $GPA^+$/TEs were cultured in serum-free Megacult-C® medium to induce their differentiation toward megakaryocytes. Representative micrographs show morphology of the $CD71^+$/TEs and $GPA^+$/TEs and BM-MNCs during 11 days of culture as determined by Wright's staining. NT-$GPA^+$ and NT-$CD71^+$ cultured under the same conditions served as controls. The images were captured by Nikon Eclipse Ti microscopy (Melville, NY, USA) using NIS Elements D4.10.00 software. Scale bar: 20 µm for all panels.

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**Figure 5:** Percentages of CD41-expressing cells generated from CD71$^+$/TEs and GPA$^+$/TEs. A-B) Expression profiles of erythroblast surface marker GPA and megakaryocyte surface marker CD41 of GPA$^+$/TEs, CD71$^+$/TEs and BM-MNCs during 11 days of culture in megakaryocyteinducing media as determined by flow cytometry. NT-GPA$^+$ and NT-CD71$^+$ cultured under the same condition served as controls. C-D) Percentages of CD41-expressing cells derived from GPA$^+$/TEs, CD71$^+$/TEs and BM-MNCs during 11 days of culture in megakaryocyte-inducing media as determined by flow cytometry. NT-GPA$^+$ and NT CD71$^+$ cultured under the same condition served as controls. Data were presented as mean ± SD of three independent experiments. The Mann-Whitney U test was used to assess the significance of differences between the observed data. *$P \leq 0.05$.

# Transdifferentiation of erythroblasts to megakaryocytes using FLI1 and ERG transcription factors

*FLI1*

Relative mRNA level

0, 500, 1000, 1500, 2000, 2500, 3000, 3500

BM-MNCs | CD71$^+$/TEs | GPA$^+$/TEs | NT-GPA$^+$ | NT-CD71$^+$

*ERG*

Relative mRNA level

0, 200, 400, 600, 800, 1000, 1200, 1400

BM-MNCs | CD71$^+$/TEs | GPA$^+$/TEs | NT-GPA$^+$ | NT-CD71$^+$

*TUBB1*

Relative mRNA level

0, 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4

BM-MNCs | CD71$^+$/TEs | GPA$^+$/TEs | NT-GPA$^+$ | NT-CD71$^+$

*c-MPL*

Relative mRNA level

0, 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4

BM-MNCs | CD71$^+$/TEs | GPA$^+$/TEs | NT-GPA$^+$ | NT-CD71$^+$

**Figure 6:** Expression levels of megakaryocyte-specific genes of CD71$^+$/TEs and GPA$^+$/TEs. Graphs show relative mRNA levels of megakaryocyte-specific genes FLI1, ERG, cMPL and TUBB1 of CD71$^+$/TEs, GPA$^+$/TEs and BM-MNCs after being cultured in megakaryocyte inducing media for 11 days as determined by qRT-PCR. NT-GPA$^+$ and NT-CD71$^+$ cultured under the same condition served as controls. The expression levels of all megakaryocyte-specific genes of TEs were compared relative to the expression levels of those genes in BM-MNCs. Data are presented as mean ± SD of three independent experiments.

# Transdifferentiation of erythroblasts to megakaryocytes using FLI1 and ERG transcription factors

A.

B.

| | Phase-contrast | Fluorescence | Merge |
|---|---|---|---|
| BM-MNCs | | | |
| GPA$^+$/iMKs | | | |
| CD71$^+$/iMKs | | | |

**Figure 7:** Functional characterization of platelets derived from CD71$^+$/iMKs and GPA+/iMKs. A) Representative micrograph shows the morphology of iMK-derived platelets observed during 11 days of culture in megakaryocyte-inducing media. The images were captured by Nikon Eclipse Ti microscopy (Melville, NY, USA) using NIS Elements D4.10.00 software. Scale bar : 20 µm. B) Representative micrographs show the ability of platelets derived from CD71$^+$/iMKs, GPA$^+$/iMKs and BM-MNCs, which were labelled with fluorescent dye CFSE (green colour), to aggregate with nonlabelled human platelets in response to thrombin stimulation. 3 × $10^5$ platelets derived from GPA$^+$/iMKs and CD71+/iMKs were labelled with CFSE, mixed with an equivalent number of non-labelled human blood platelets, and treated with 0.5 U/ml thrombin to activate platelet aggregration. The images were captured by FluoViewTM FV1000 confocal microscope (Olympus). The contribution of iMK-derived platelets in the aggregrates were determined by the extent of green fluorescence. Scale bar: 20 µm.