MICROGRAVITY AFFECTS CLUSTER FORMATION, DIFFERENTIATION, AND CYTOSKELETAL ORGANIZATION OF RAUSCHER MURINE ERYTHROLEUKEMIA CELLS

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Abstract

Humans and experimental animals subjected to microgravity, such as experienced during space flight, exhibit alterations in erythropoiesis, including changes in red blood cell morphology, survival and reduction in red blood cell mass. Human bone marrow cells grown on orbit showed a profound reduction in numbers of erythroid cells. We now report results of a study carried out on orbit (ISS UF-1) in which an erythroid cell line was induced to differentiate. Rauscher murine erythroleukemia cells, a continuous cell line that can undergo erythropoietin (Epo)- or chemical-induced differentiation similar to normal erythropoiesis, were cultured for 6 days either in microgravity or on earth and then for 3 days in the absence or presence of 50 U Epo/ml or 0.7% dimethylsulfoxide (DMSO). The cells were then fixed, stored on orbit and returned to earth for study. Compared to ground-based controls, cells cultured in microgravity exhibited a significantly higher percentage of cluster formation ($p<0.05$) and a greater degree of differentiation (hemoglobinization) ($p<0.01$). Actin content appeared reduced in microgravity, and following Epo or DMSO treatment, there was a more profound loss of actin stress fibers in microgravity. These results are consistent with the hypothesis that erythropoiesis is affected by gravitational forces at the cellular level.

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Introduction

"Anemia of spaceflight" is an abnormality resulting from microgravity exposure both in humans and in experimental animals. Although alterations in hematopoiesis and red cell survival have been implicated in this phenomenon, the experimental data are not always easy to interpret due to the complex physiological response of the body to space flight and to the complex hormonal interplay on hematopoiesis.

Cell-culture based model systems make it simpler to observe the direct effect(s) of microgravity on hematopoietic cells. In the present study, we used Rauscher murine erythroleukemia cells (Rauscher cells) to study cell growth and differentiation in space. Rauscher cells undergo hemoglobinization, a marker of erythroid cell differentiation, in the presence of inducing agents such as DMSO and Epo. Rauscher cells also express other differentiation antigens that make them useful models.

The objective of the present research is to study the effects of microgravity on:

1) **cell-cell association**
2) **cell cytoskeletal organization**
3) **cell differentiation.**

Materials and Methods

Rauscher cells were cultured for 3 days in the International Space Station (ISS) (under microgravity) or on earth (under unit gravity) without (non-induced) or with (induced) either 50 U Epo /mL or 0.7% DMSO and then fixed in formalin.

Hemoglobin was stained by benzidine-staining method.

Actins were stained by fluorescent-conjugated phalloidin or deoxyribo-nuclease I.

Cell membrane antigens were stained by fluorescent-conjugated antibodies.

Images were captured by an Olympus digital camera and processed by Microsuite software♦.

♦We are grateful to Aid for Cancer Research for their generous gift of an Olympus digital camera and Microsuite software.

Microgravity promotes formation of tight cell aggregates

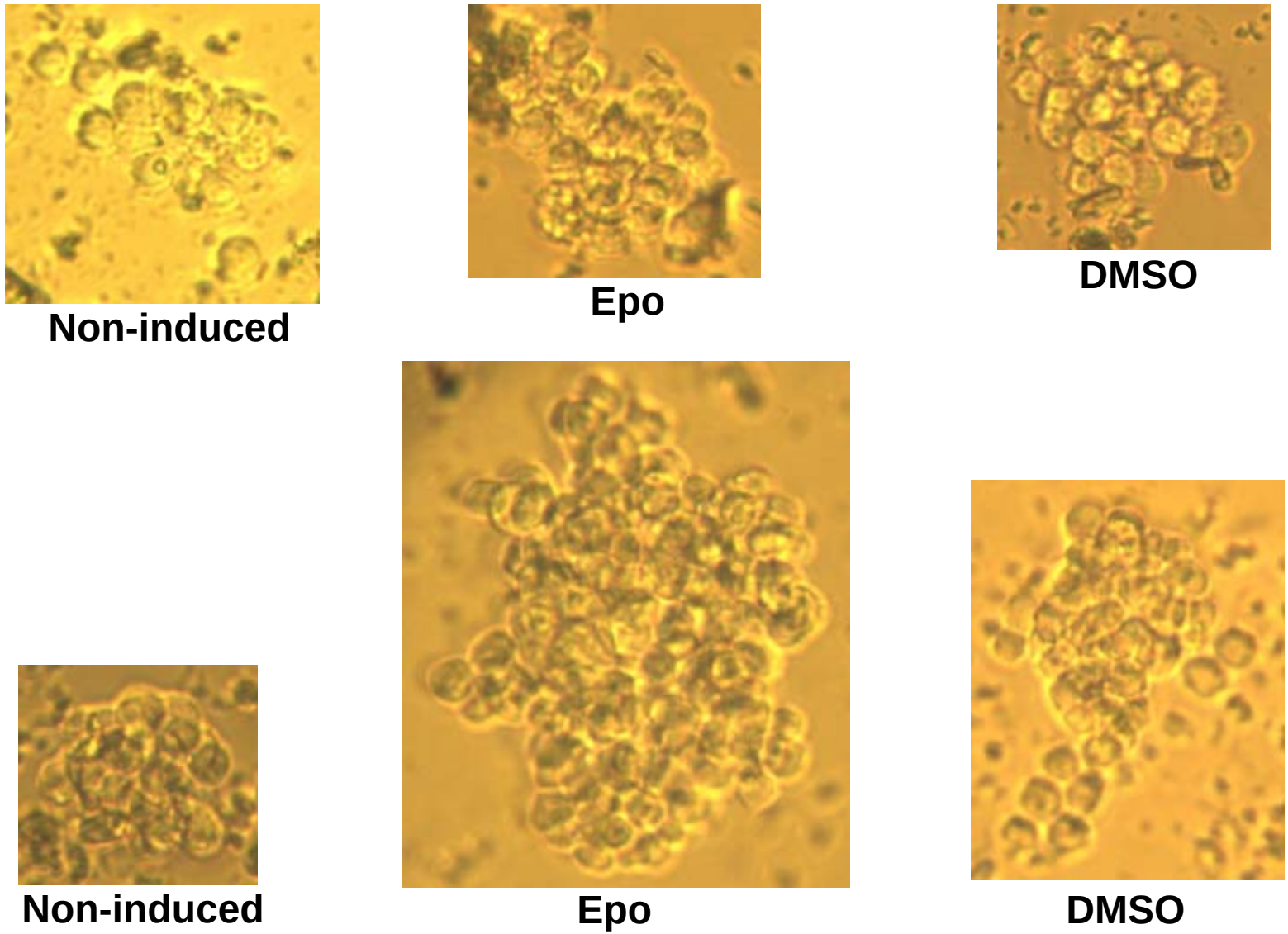


Figure 1. Bright-field images of fixed Rauscher cells. Under unit gravity (top), the cells formed small and loose clusters, while under microgravity (bottom), the cells formed large and compact clusters.

Microgravity promotes cell-cell association

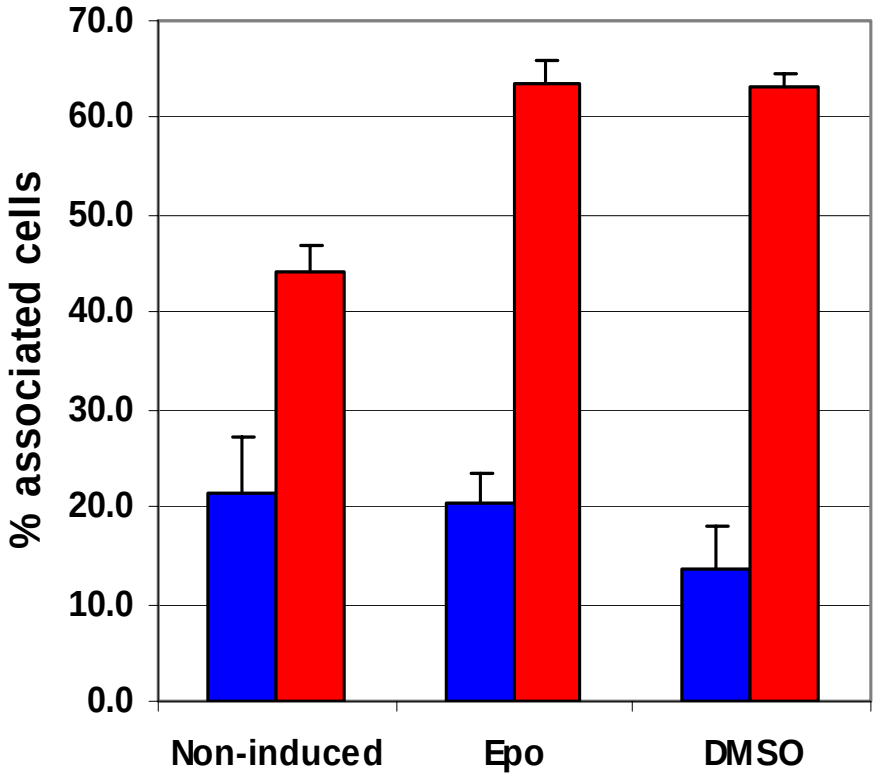


Figure 2. Rauscher cells were cultured under either unit gravity (■) or microgravity (■) with or without induction by DMSO or Epo. In all instances, the percentage of associated cells under microgravity was significantly greater than under unit gravity.

Microgravity promotes large cell cluster formation

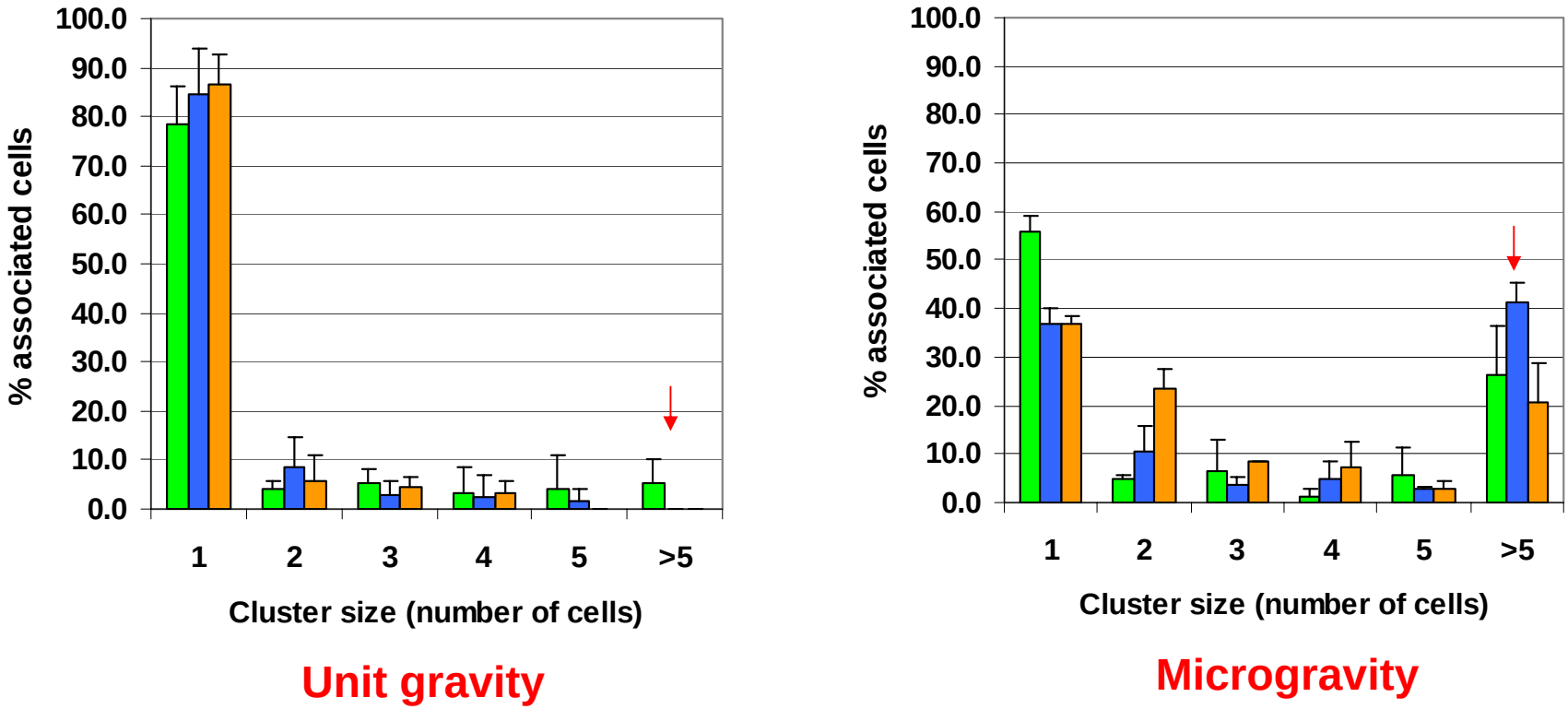


Figure 3. Rauscher cells were cultured under either unit gravity or microgravity without (■), or with Epo (■) or DMSO (■). Significantly more larger cell clusters (> 5 cells, ↓) were present in the culture under microgravity (right panel) than under unit gravity (left panel).

Microgravity alters G-actin expression

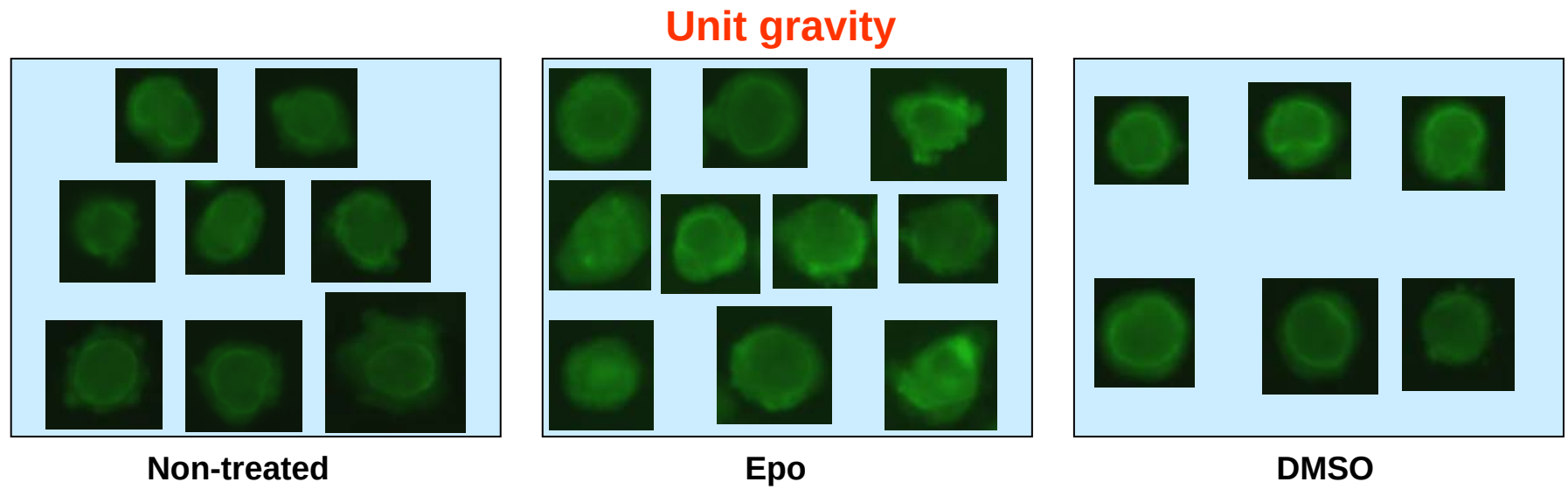


Figure 4. Fluorescent images of G-actin in Rauscher cells stained by Alexa Fluor-488 deoxyribonuclease I. G-actin was higher in non-induced but lower in induced cells cultured under microgravity (bottom) than under unit gravity (top).

Microgravity reduces F-actin expression

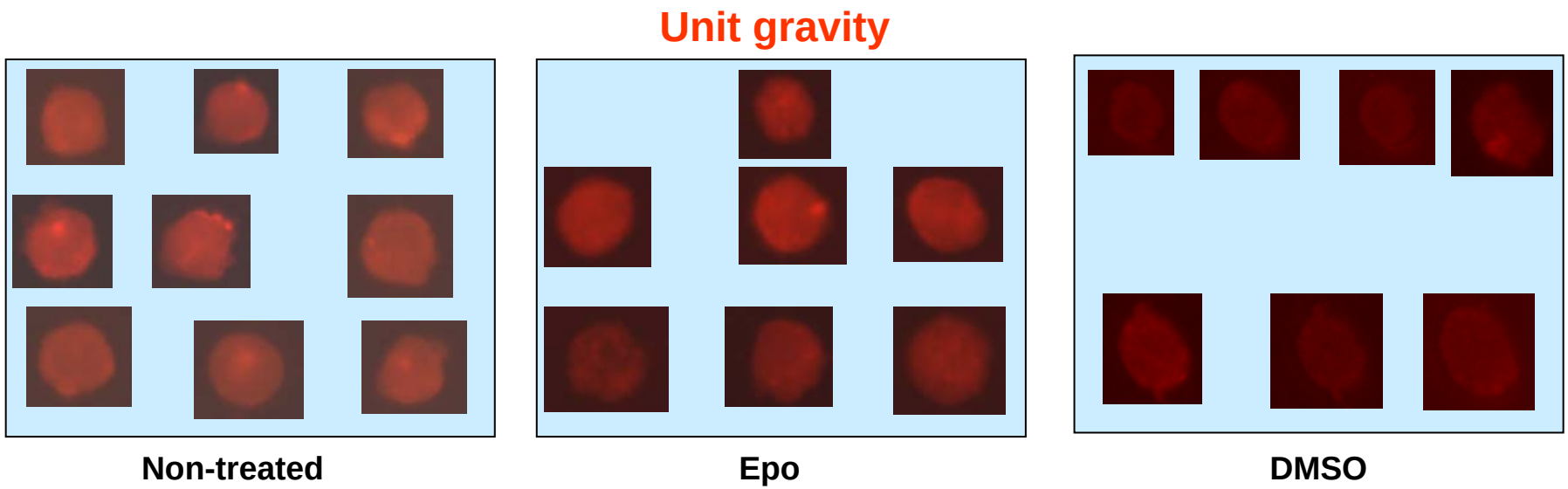


Figure 5: Fluorescent images of F-actin in Rauscher cells stained by Alexa Fluor-568 phalloidin. F-actin was low in cells cultured under microgravity (bottom) but high under unit gravity (top).

Microgravity stimulates hemoglobin synthesis

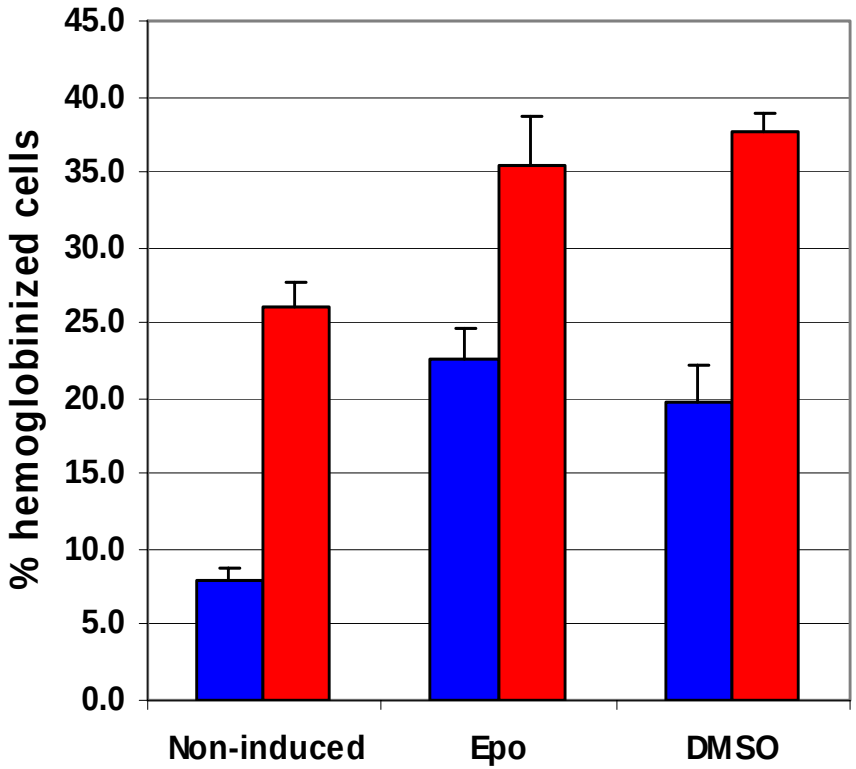


Figure 6. Rauscher cells were cultured under either unit gravity (■) or microgravity (■) with or without induction by Epo or DMSO. In all instances, the percentage of hemoglobinized cells under microgravity was significantly greater than under unit gravity. Note high degree of spontaneous hemoglobinization in microgravity without added inducer.

Microgravity increases glycophorin A expression

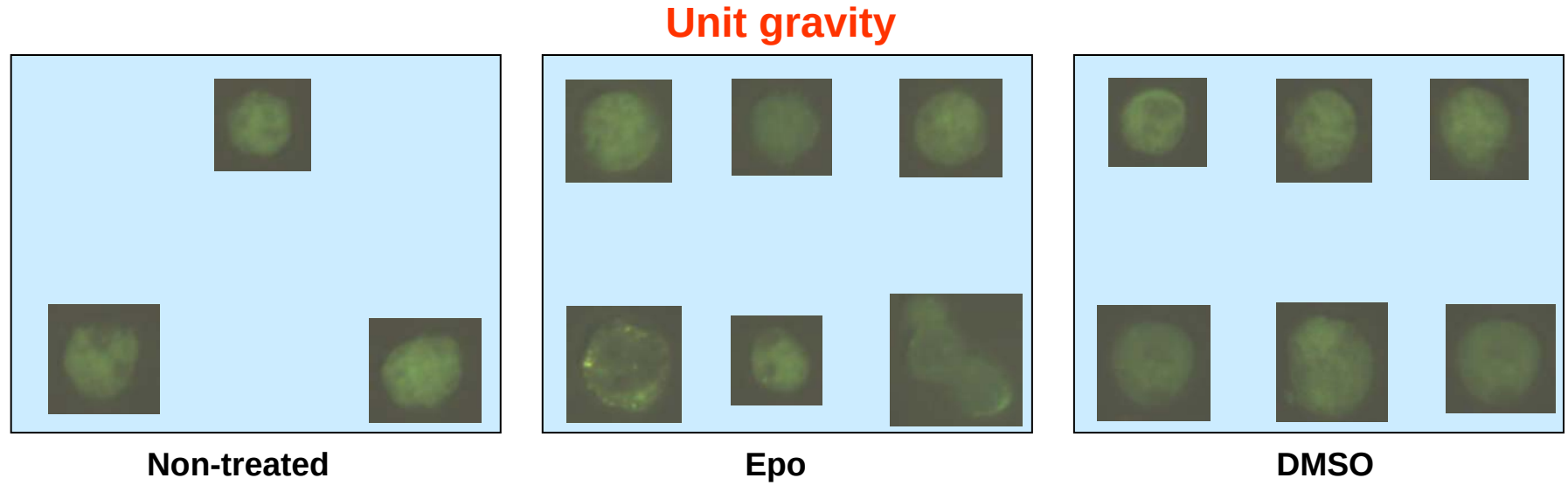


Figure 7: Fluorescent images of glycophorin A on Rauscher cells stained by mouse anti-human glycophorin A (α) & goat anti-mouse IgM-FITC. Glycophorin A signal was strong in cells cultured under microgravity (bottom) but weak under unit gravity (top).

Microgravity reduces TER-119* expression

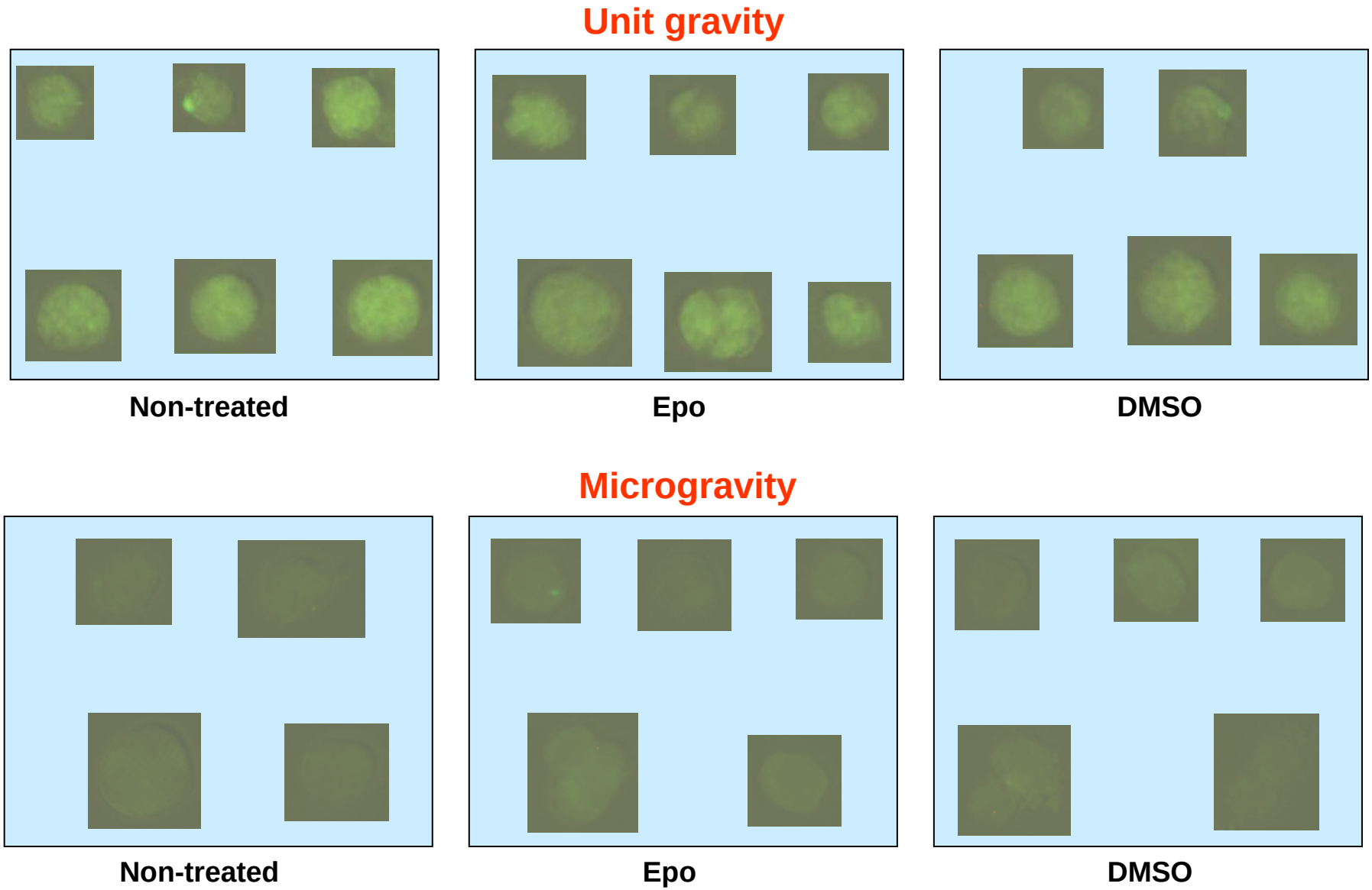


Figure 8: Fluorescent images of TER-119 on Rauscher cells stained by biotinylated rat anti-mouse TER-119 & streptavidin-FITC. TER-119 was absent in cells cultured under microgravity (bottom) but present under unit gravity (top).

* **The TER-119 is:**

- ✪ **a mouse erythroid cell marker and present from early proerythroblast to mature erythrocyte**
- ✪ **absent from cells carrying BFU-E and CFU-E activities**

(Kina. T., et al., 2000; Vanucchi, AM., et al., 2000)

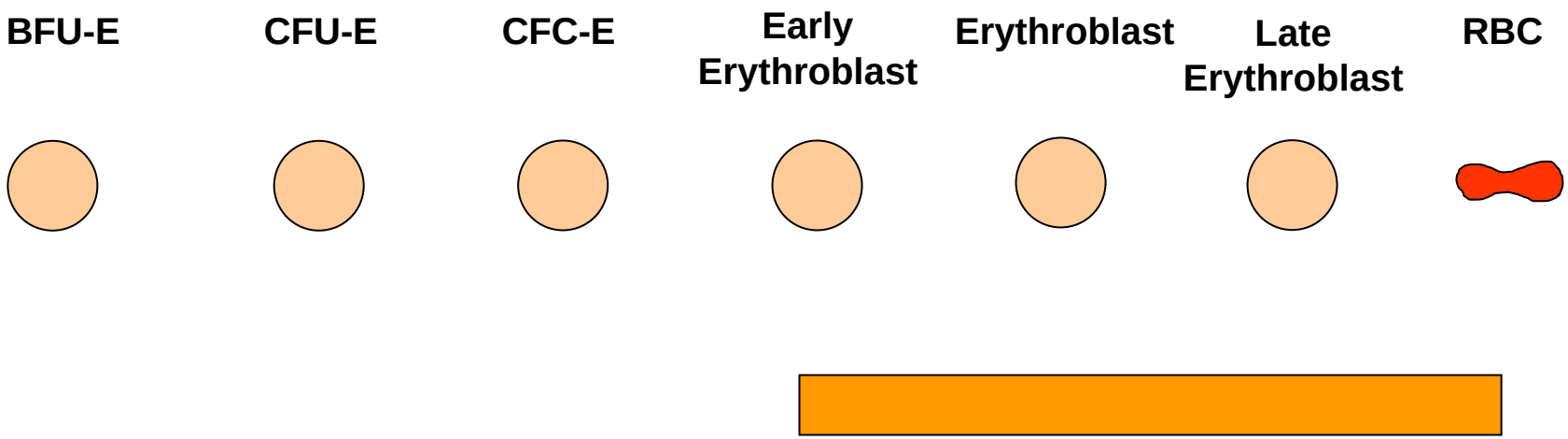
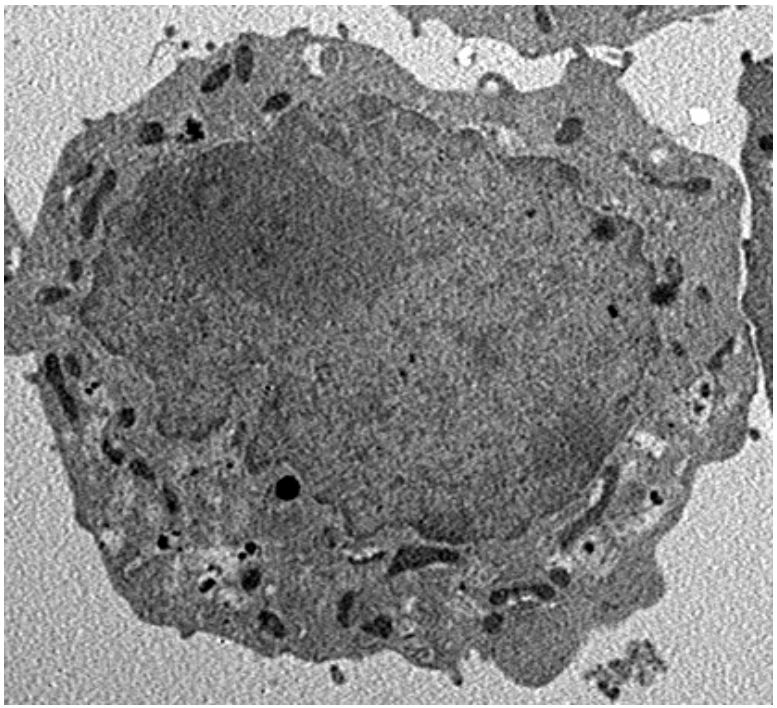
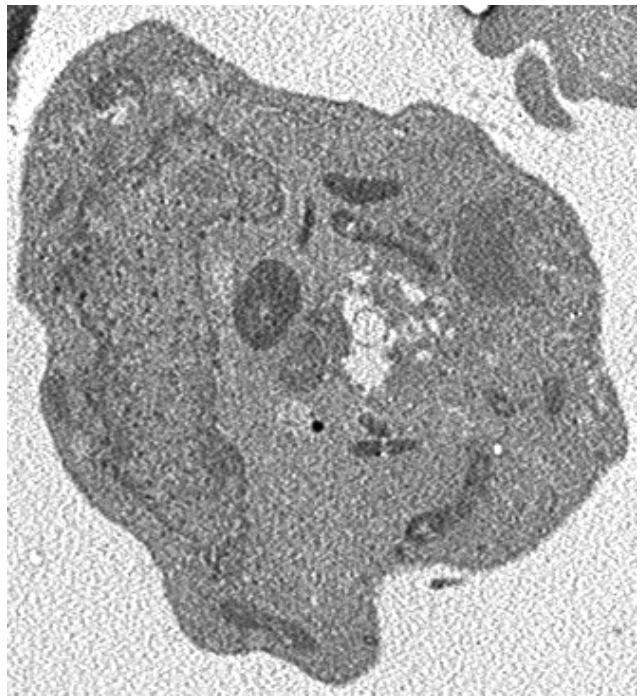


Figure 9. Model of TER-119 expression during normal erythropoiesis.

Microgravity promotes the reduction of mitochondria



Unit gravity



Microgravity

Figure 10. Transmission Electron Micrographs of Rauscher cells. Fewer mitochondria are present in the cells cultured under microgravity (right) than under unit gravity (left).

Table. EMSIII cell phenotypes under microgravity and under unit gravity (summary)

Targets	Microgravity	Unit gravity
Cell cluster	Large, compact	Small, loose
G-actin	↑ (non-induced) ↓ (Induced)	↓ (non-induced) ↑ (Induced)
F-actin	↓	↑
Hemoglobin	↑	↓
Glycophorin A	↑	↓
TER-119	↓	↑
Mitochondrion	↓	↑
Ankyrin B (not shown)	No change	No change
β-spectrin (not shown)	No change	No change

Conclusions

In parallel studies conducted in our ground-based laboratory and under conditions of microgravity aboard the International Space Station (ISS), we observed that microgravity had selected profound effects on cultured cells.

Using Rauscher murine erythroleukemia cells as a model of erythroid progenitor cells, we observed that

1. Microgravity affected the aggregation, or clustering, of the cells in culture.

2. Microgravity affected the cytoskeletal organization, as indicated by alterations in G-actin and decreases in F-actin staining.

3. Microgravity increased cell differentiation, in the absence of chemical or hormonal inducers.

4. Cell differentiation under microgravity conditions is abnormal, as indicated by the absence of TER-119.