Elucidating the Potential Roles of E-cadherin in Erythroid and Megakaryocyte Differentiation

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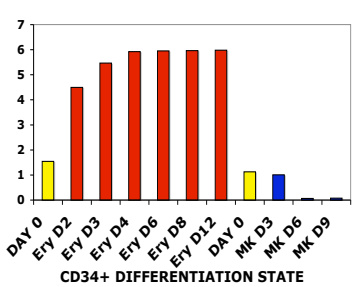
ABSTRACT

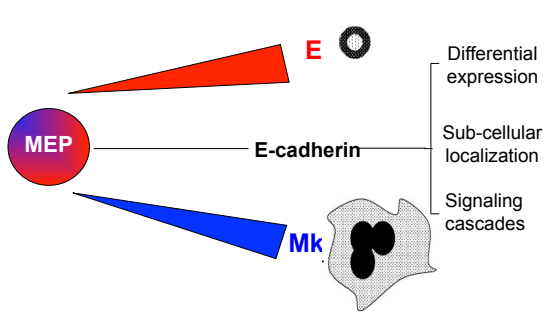
E-cadherin is a transmembrane receptor that mediates cell-cell interactions and has crucial roles in epithelial cell organization, and tumorigenesis. Apart from one early study reporting a potential function for E-cadherin in erythropoiesis, its role in hematopoiesis has not been investigated. Using RNA-sequencing, we show differential expression of E-cadherin during megakaryocyte (Mk) and erythroid (E) differentiation, suggesting a potential novel role for E-cadherin in the fate decision of the Mk-E-progenitor (MEP). Consistent with a potential role for E-cadherin in erythropoiesis, we find that an E-cadherin inhibitory antibody blocks erythroid differentiation of primary human peripheral blood CD34$^+$ cells, causing a 50% reduction in expression of erythroid-specific markers (GYPA, CD71) by flow cytometry. Also, in primary murine fetal liver erythroid cultures, E-cadherin inhibition increases the % of cells in the most immature erythroid state. In assessing a role for E-cadherin in Mks, we have found that Mks in mouse bone marrow and fetal liver express E-cadherin. Immunostaining reveals that subcellular localization of E-cadherin in bone marrow Mks is diffusely cytoplasmic, in contrast to its concentration at the plasma membrane in epithelial cells. Using intracellular flow cytometry, we find CD41$^+$ Mks with intracellular E-cadherin staining. In western blot analysis of murine bone marrow and fetal liver Mk cultures, a smaller isoform of E-cadherin is enriched in more mature Mks, which may be derived from a proteolytic cleavage event. We have recently generated a mouse model with Mk-specific deletion of E-cadherin, and preliminary analyses of heterozygous E-cadherin deleted adult mice show no significant differences in ploidy or platelet counts, but 2 of 5 animals display splenic abnormalities, which correlate with enhanced iron deposition. In contrast, in fetal liver Mks, we find that E-cadherin deletion may result in decreased ploidy and an increase in the % of Mk progenitors, but this needs further investigation. We anticipate that data generated through this project will extend the current knowledge of the role of cadherins in hematopoiesis.

HYPOTHESIS

E-cadherin is differentially expressed in human megakaryocyte and erythroid lineages during differentiation of CD34+ cells

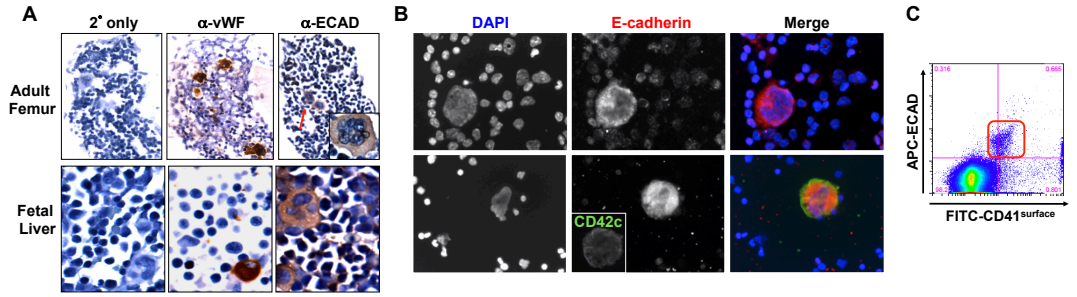


Human hematopoietic CD34$^+$ progenitor cells expanded for 6 days (Day 0; yellow bars) were induced to differentiate down the E lineage with EPO (red bars), and the Mk lineage with TPO (blue bars). At various time points during differentiation, total RNA was harvested and cDNA was generated and subjected to sequencing. Day 0 represents two different cell expansions, each composed of a heterogeneous pool of progenitor cells.



RESULTS

FIGURE 1. E-cadherin is expressed in fetal liver & adult megakaryocytes and is primarily intracellular

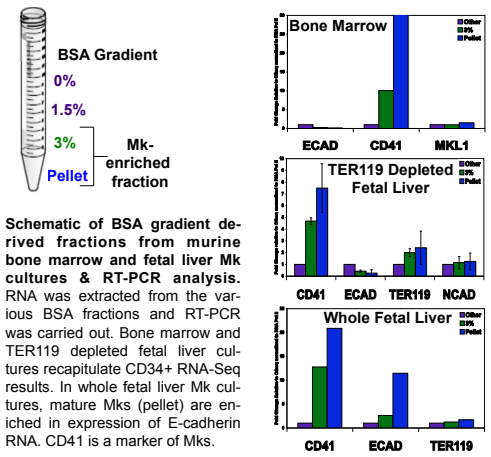


A. Murine adult bone marrow and fetal liver megakaryocytes express E-cadherin. Immunohistochemistry on paraffin-embedded sections using an E-cadherin primary antibody (ProteinTech). Red arrow indicates a Mk staining positive for E-cadherin.

B. E-cadherin staining colocalizes with CD42c. Immunofluorescence on murine bone marrow cells identifies large polyploid Mks with CD42c membrane staining which costain with E-cadherin.

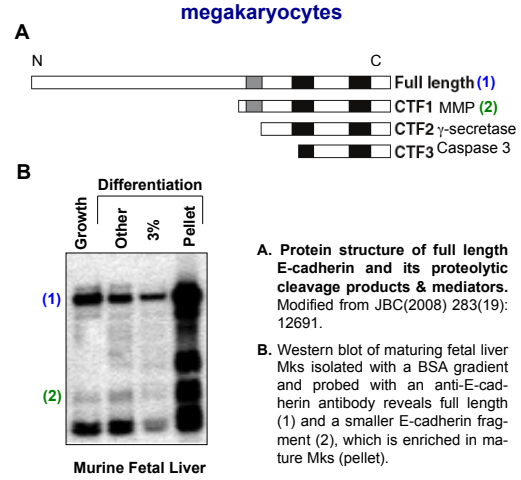
C. A population of CD41$^{+Surface}$ECAD^{+Intra} cells exist in murine bone marrow. Double positive CD41$^{+Surface}$ECAD^{+Intra} cells are circled red.

FIGURE 2. E-cadherin RNA levels decrease during Mk maturation in bone marrow but is enriched in mature fetal Mks



Schematic of BSA gradient derived fractions from murine bone marrow and fetal liver Mk cultures & RT-PCR analysis. RNA was extracted from the various BSA fractions and RT-PCR was carried out. Bone marrow and TER119 depleted fetal liver cultures recapitulate CD34+ RNA-Seq results. In whole fetal liver Mk cultures, mature Mks (pellet) are enriched in expression of E-cadherin RNA. CD41 is a marker of Mks.

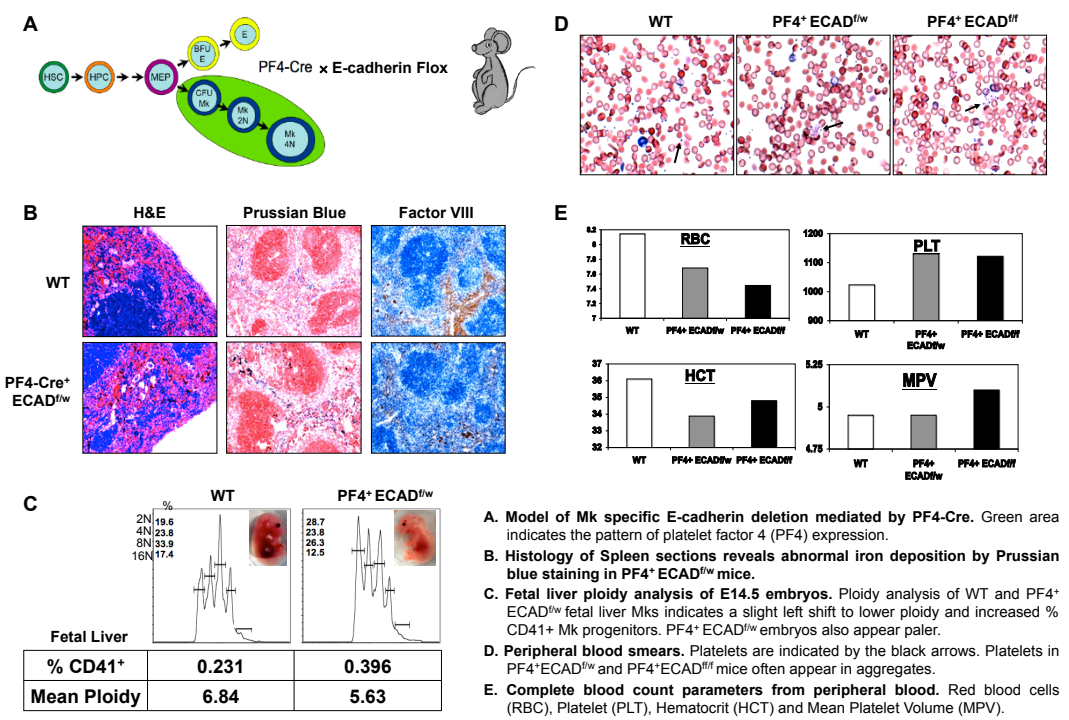
FIGURE 3. Proteolytic cleavage products of E-cadherin are enriched in maturing megakaryocytes



A. Protein structure of full length E-cadherin and its proteolytic cleavage products & mediators. Modified from JBC(2008) 283(19): 12691.

B. Western blot of maturing fetal liver Mks isolated with a BSA gradient and probed with an anti-E-cadherin antibody reveals full length (1) and a smaller E-cadherin fragment (2), which is enriched in mature Mks (pellet).

FIGURE 4. Analysis of a mouse model of megakaryocyte specific deletion of E-cadherin



A. Model of Mk specific E-cadherin deletion mediated by PF4-Cre. Green area indicates the pattern of platelet factor 4 (PF4) expression.

B. Histology of Spleen sections reveals abnormal iron deposition by Prussian blue staining in PF4$^+$ ECAD$^{f/w}$ mice.

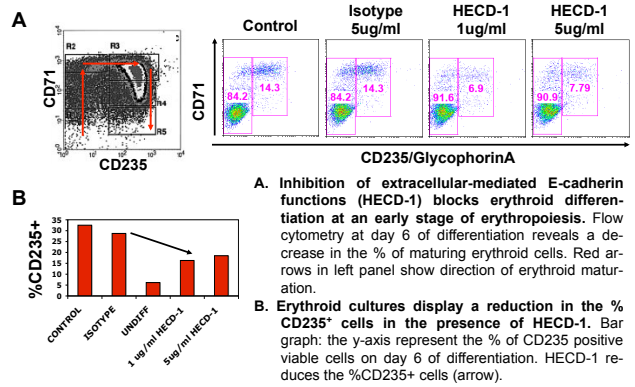
C. Fetal liver ploidy analysis of E14.5 embryos. Ploidy analysis of WT and PF4$^+$ ECAD$^{f/w}$ fetal liver Mks indicates a slight left shift to lower ploidy and increased % CD41+ Mk progenitors. PF4$^+$ ECAD$^{f/w}$ embryos also appear paler.

D. Peripheral blood smears. Platelets are indicated by the black arrows. Platelets in PF4$^+$ECAD$^{f/w}$ and PF4$^+$ECAD$^{f/f}$ mice often appear in aggregates.

E. Complete blood count parameters from peripheral blood. Red blood cells (RBC), Platelet (PLT), Hematocrit (HCT) and Mean Platelet Volume (MPV).

RESULTS

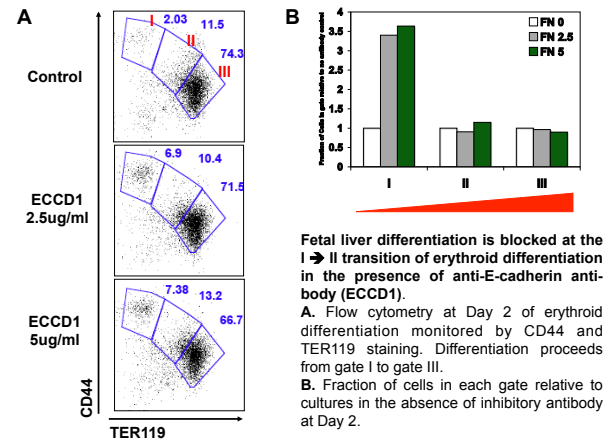
FIGURE 5. Inhibition of E-cadherin extracellular mediated functions blocks erythroid differentiation of human CD34+ peripheral blood stem cells



A. Inhibition of extracellular-mediated E-cadherin functions (HECD-1) blocks erythroid differentiation at an early stage of erythropoiesis. Flow cytometry at day 6 of differentiation reveals a decrease in the % of maturing erythroid cells. Red arrows in left panel show direction of erythroid maturation.

B. Erythroid cultures display a reduction in the % CD235$^+$ cells in the presence of HECD-1. Bar graph: the y-axis represent the % of CD235 positive viable cells on day 6 of differentiation. HECD-1 reduces the %CD235+ cells (arrow).

FIGURE 6. Murine Fetal Liver erythropoiesis is blocked at early stages of erythroid differentiation with an anti-E-cadherin antibody



Fetal liver differentiation is blocked at the I → II transition of erythroid differentiation in the presence of anti-E-cadherin antibody (ECCD1).

A. Flow cytometry at Day 2 of erythroid differentiation monitored by CD44 and TER119 staining. Differentiation proceeds from gate I to gate III.

B. Fraction of cells in each gate relative to cultures in the absence of inhibitory antibody at Day 2.

SUMMARY & FUTURE DIRECTIONS

Megakaryocytes:

- Mks express E-cadherin intracellularly
  - Determine the precise localization of E-cadherin within Mks (nuclear, endosomal)
- E-cadherin proteolytic cleavage fragments are abundant in mature Mks
  - Identify which fragments are expressed and which cleavage events are responsible for these species
  - Investigate potential functions of intracellular/nuclear E-cadherin during Mk differentiation (transcription of cyclins, MMPs)
- Mk specific deletion of E-cadherin may cause bleeding problems in adult mice and reduces the abundance of mature Mks in fetal hematopoiesis
  - Continue characterization of Mks & platelets from fetal & adult mice

Erythroid Lineage:

- Inhibition of extracellular E-cadherin blocks erythroid differentiation at early stages of erythropoiesis
  - Elucidate which downstream binding partners and signaling pathways involving E-cadherin are necessary to promote erythroid differentiation

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