Exploring the Role of E-cadherin in Megakaryopoiesis, Platelet Biogenesis and Hemostasis

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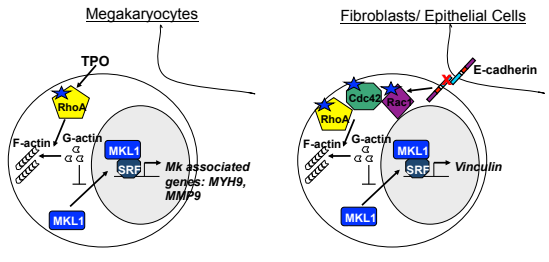
ABSTRACT

Epithelial (E-) cadherin is an adhesion molecule that mediates cell-cell interactions, and is important in pluripotent stem cell reprogramming. We are investigating the role of E-cadherin in megakaryocyte (Mk) differentiation and platelet function. We propose that E-cadherin mediates interactions that facilitate the essential roles of platelets, including thrombus formation, and also engages in critical signaling pathways to promote megakaryopoiesis. We have evidence that mature Mks and platelets express E-cadherin. Our data indicate an intracellular pool of E-cadherin within CD41/42+ Mks. Given that E-cadherin displays dynamic translational regulation during Mk differentiation, we generated Mk-specific E-cadherin knockout mice to investigate E-cadherin function in this program. The Mk-specific PF4-Cre strain was bred to E-cadherin floxed mice to produce the knockout. Heterozygous and homozygous E-cadherin deleted mice are viable and fertile, with normal platelet counts. The spleens of E-cadherin deleted mice often display hemosiderin-rich regions, suggestive of extravasation of RBCs. To test if these mice have a bleeding defect, bleeding times were performed. Preliminary data suggest that E-cadherin deletion increases bleeding time in the heterozygous (Ecadf/w:85±8secs) and homozygous (Ecadf/f: 69±9secs) state relative to wild type (Ecadw/w:57±9secs). Platelet immunofluorescence suggests that loss of E-cadherin disrupts tubulin architecture and overall platelet morphology. Future studies will focus on elucidating what aspects of platelet function are defective in the absence of E-cadherin. Initial studies using an E-cadherin blocking antibody in human platelets shows potential impairment of aggregation in response to ADP and collagen. We are also examining how loss of E-cadherin affects differentiation prior to platelet formation, and preliminary data indicate that E-cadherin deletion reduces fetal liver Mk ploidy, with an accumulation of immature low ploidy Mks. The data generated through this project will highlight novel non-epithelial roles for E-cadherin, and will enlighten our understanding of the molecular aspects of platelet interactions during thrombus formation.

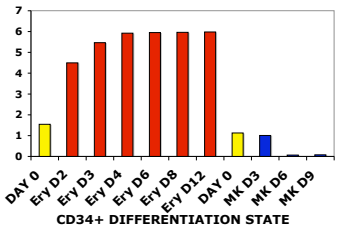
HYPOTHESIS and RATIONALE

Hypothesis: E-cadherin functions in megakaryopoiesis, platelet formation, and hemostasis.

- RhoGTPase activation is required upstream of MKL1/SRF signaling to promote megakaryopoiesis (Smith, Teixeira et al., Blood 2013)
- MKL1 knockout mice & Mk-specific SRF knockout mice have ↓ platelet counts and impaired megakaryopoiesis (Cheng et al., Blood 2009; Halene et al., 2010)
- Disintegration of E-cadherin junctions activates MKL1/SRF signaling via Rac/Rho activation in fibroblasts/epithelial cells (Busche et al., J Cell Sci. 2008, 2010)
- A cleaved isoform of E-cadherin translocates to the nucleus & induces expression of MMPs and cyclins (Salahshor et al., Mod.Path. 2008, Ferber et al., JBC 2008)
  - MMP9 is a MKL1/SRF target gene; Cyclin D1 regulates Mk polyploidization
- E-cadherin (RNA) is differentially expressed in human megakaryocyte and erythroid lineages during differentiation of CD34+ cells (unpublished)



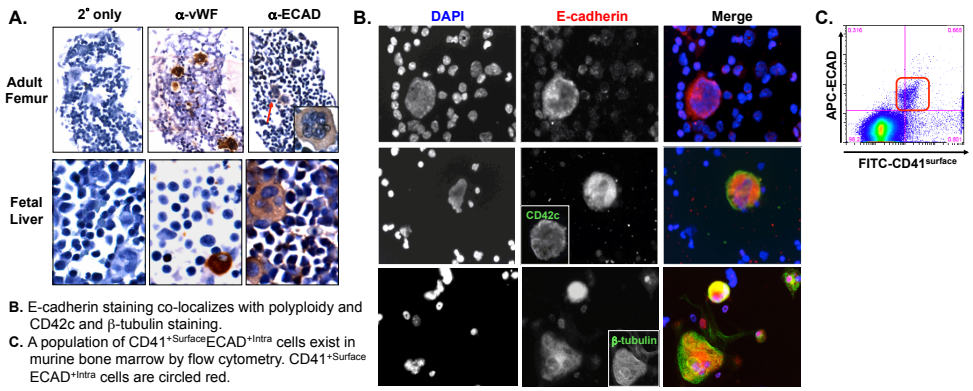
E-cadherin is differentially expressed in human megakaryocyte and erythroid lineages during differentiation of CD34+ cells.



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

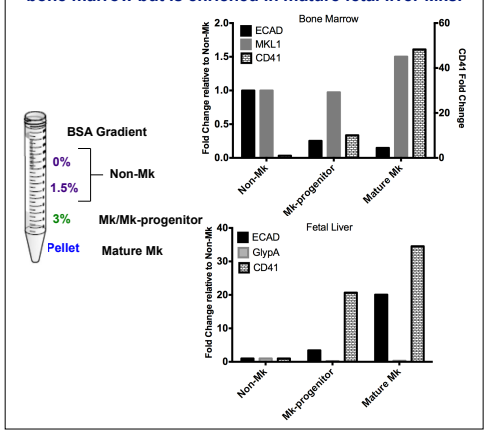
RESULTS

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

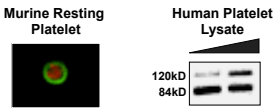


B. E-cadherin staining co-localizes with polyploidy and CD42c and β-tubulin staining.
C. A population of CD41$^{+Surface}$ECAD^{+Intra} cells exist in murine bone marrow by flow cytometry. CD41$^{+Surface}$ ECAD^{+Intra} cells are circled red.

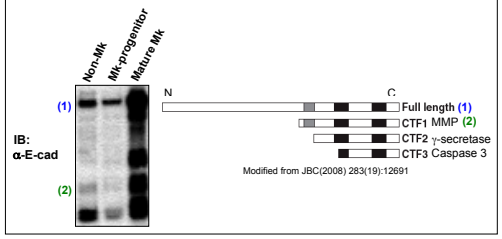
E-cadherin RNA levels decrease during Mk maturation in bone marrow but is enriched in mature fetal liver Mks.



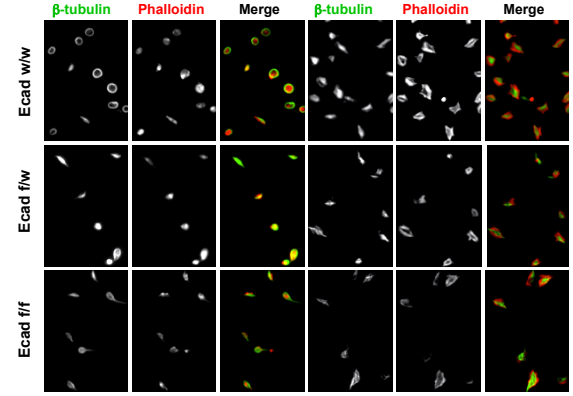
E-cadherin is expressed in murine and human platelets.



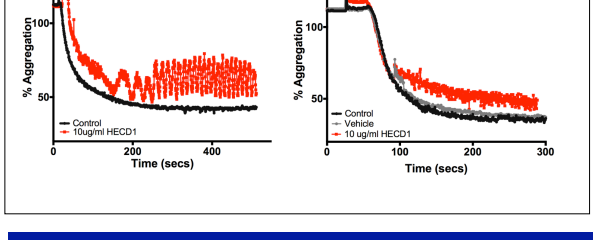
Proteolytic cleavage products of E-cadherin are enriched in mature fetal liver Mks.



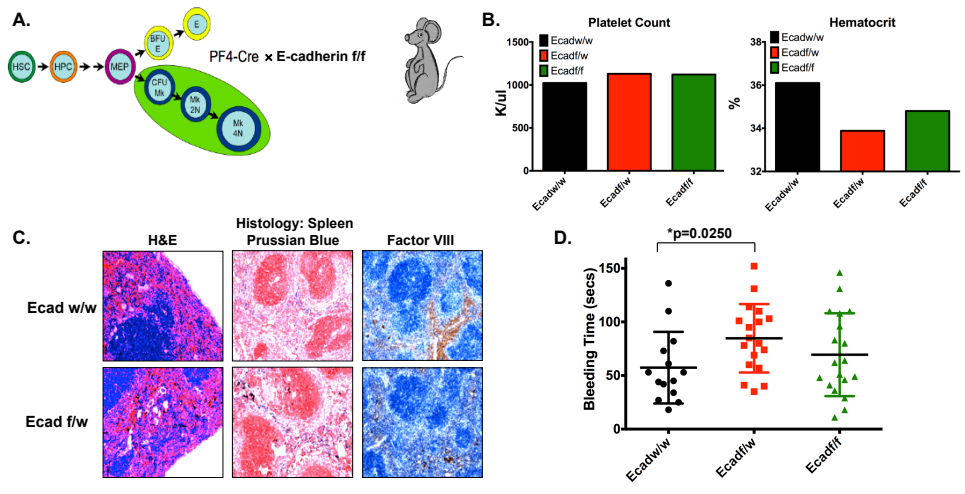
E-cadherin null platelets have impaired morphology in the resting state but can still undergo spreading when activated.



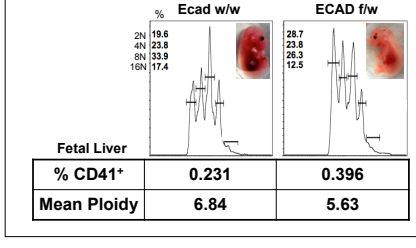
E-cadherin blocking antibody - HECD1 - partially inhibits human platelet aggregation in response to ADP and Collagen.



Megakaryocyte specific deletion of E-cadherin results in a bleeding defect as indicated by histology (hemosiderin deposition in the spleen), decreased hematocrit, and bleeding times, without affecting platelet counts.

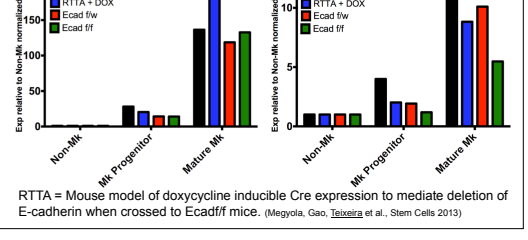


Loss of E-cadherin in fetal liver Mks causes an accumulation of low ploidy CD41+ Mks with a subsequent decrease in overall ploidy.



% CD41⁺	0.231	0.396
Mean Ploidy	6.84	5.63

Loss of E-cadherin in Mks impairs the expression of genes associated with megakaryopoiesis.



RTTA = Mouse model of doxycycline inducible Cre expression to mediate deletion of E-cadherin when crossed to Ecadf/f mice. (Megyola, Gao, Teixeira et al., Stem Cells 2013)

SUMMARY and FUTURE DIRECTIONS

- Mks and platelets express E-cadherin.
  - Determine the precise localization of E-cadherin within Mks.
- E-cadherin proteolytic cleavage fragments are abundant in mature Mks.
  - Identify which fragments are expressed.
  - Investigate potential functions of intracellular/nuclear E-cadherin during Mk differentiation.
- Mk specific deletion of E-cadherin may cause bleeding abnormalities without affecting platelet counts.
  - Quantify blood volume loss (hemoglobin).
- Loss of E-cadherin in fetal liver Mks impairs differentiation and results in an accumulation of immature progenitors.
- E-cadherin may be required to promote some Mk-associated gene programs.
- Platelets lacking E-cadherin exhibit morphological abnormalities.
  - Investigate ultrastructure and granular composition of E-cadherin null platelets.
- Platelet aggregation is partially impaired by the use of E-cadherin blocking antibodies.
  - Characterize the aggregation capacity of the E-cadherin null platelets.

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