Endoglin has a crucial role in blood cell-mediated vascular repair

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NIH workshop – HHT Bethesda, 8th June 2006

Background

- Endoglin: accessory receptor for TGF-β in vascular endothelial cells (EC)
  - Essential for angiogenesis (mouse development)
- Hereditary hemorrhagic telangiectasia type 1 (HHT1): mutations endoglin gene
  - Vascular malformations increasing with age
  - Haploinsufficiency
  - Clinical manifestations variable

Background

- Mononuclear cells (MNCs) can express endoglin
  - Endothelial progenitor cells, circulating EC, bone marrow monocytic lineages
- MNCs contribute to vascular repair
  - Transdifferentiation to EC; vasculogenesis
  - Secretion growth factors and cytokines; angiogenesis

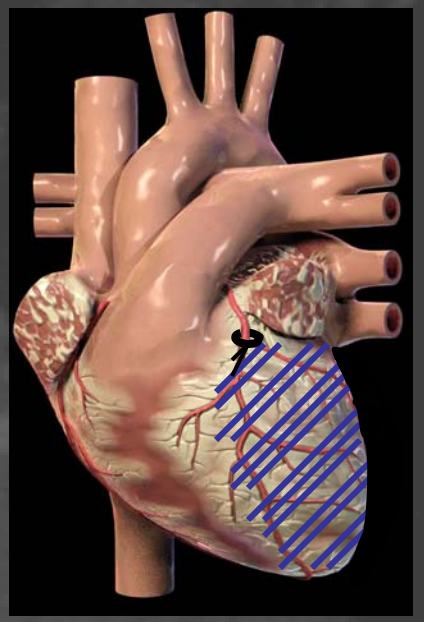
Hypothesis

- Vascular repair, mediated by MNCs, may be impaired in subjects with HHT1

- Model
  - HHT1 patients and mice for endoglin mutation
  - Myocardial infarction (MI) for angiogenesis and vasculogenesis

MI in mice

- Myocardial infarction
  or
- Sham thoracotomy



- 4 week survival >60%

- Endoglin heterozygous mice (*Eng*$^{+/-}$)
- Wildtype littermates (*Eng*$^{+/+}$)
- Wildtype Balb/C

MNC injection

- Venous blood from HHT1 patients or healthy volunteers

MNC injection

- Venous blood from HHT1 patients or healthy volunteers
- Density gradient centrifugation → MNC population
- 1-3 hours after MI 5 million cells (or PBS) in tail vein
- Tacrolimus (Prograft®) for immunosuppression

Analysis

- Endoglin expression infarcted *vs.* healthy heart (human and mouse): ISH and IHC

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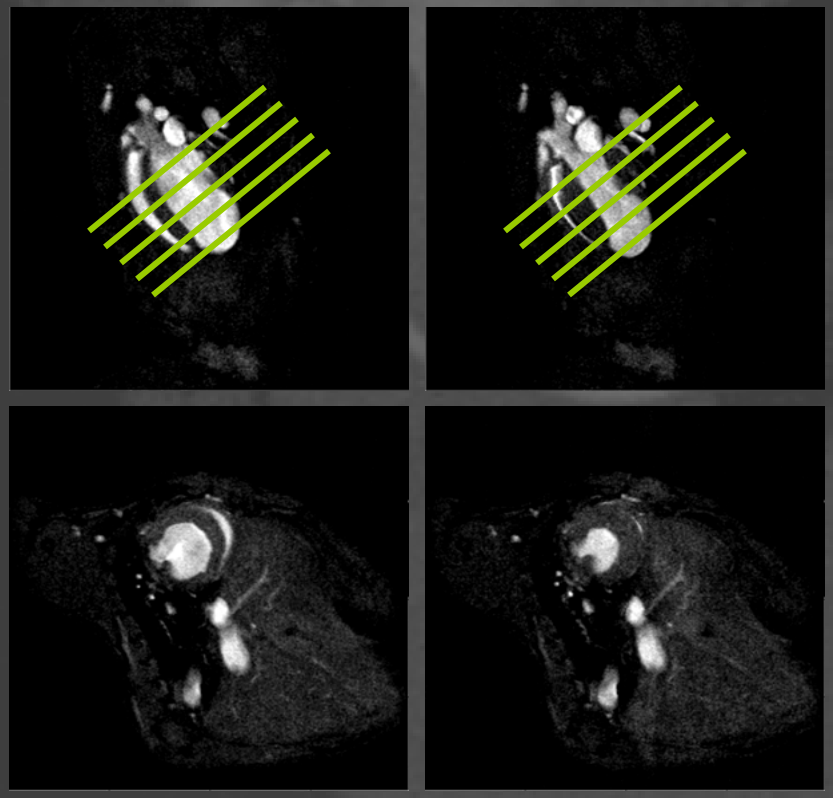
- Heart function: mouse MRI (1w, 4w)

Magnetic Resonance Imaging

4 Chamber

Short axis



End Diastolic Diameter (EDD)

End Systolic Diameter (ESD)

Ejection Fraction (EF)= (EDD-ESD) / EDD

Cardiac index = EF*EDD*heart rate / weight

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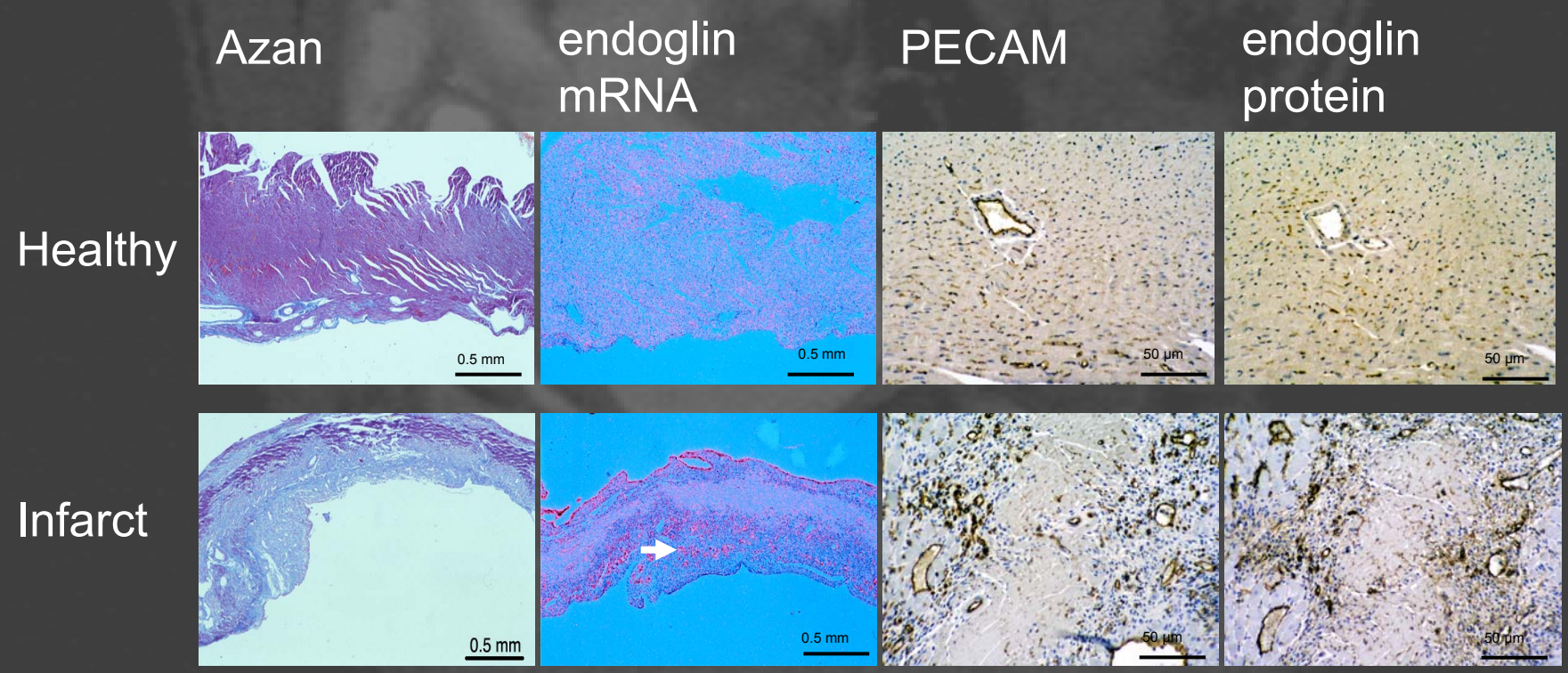
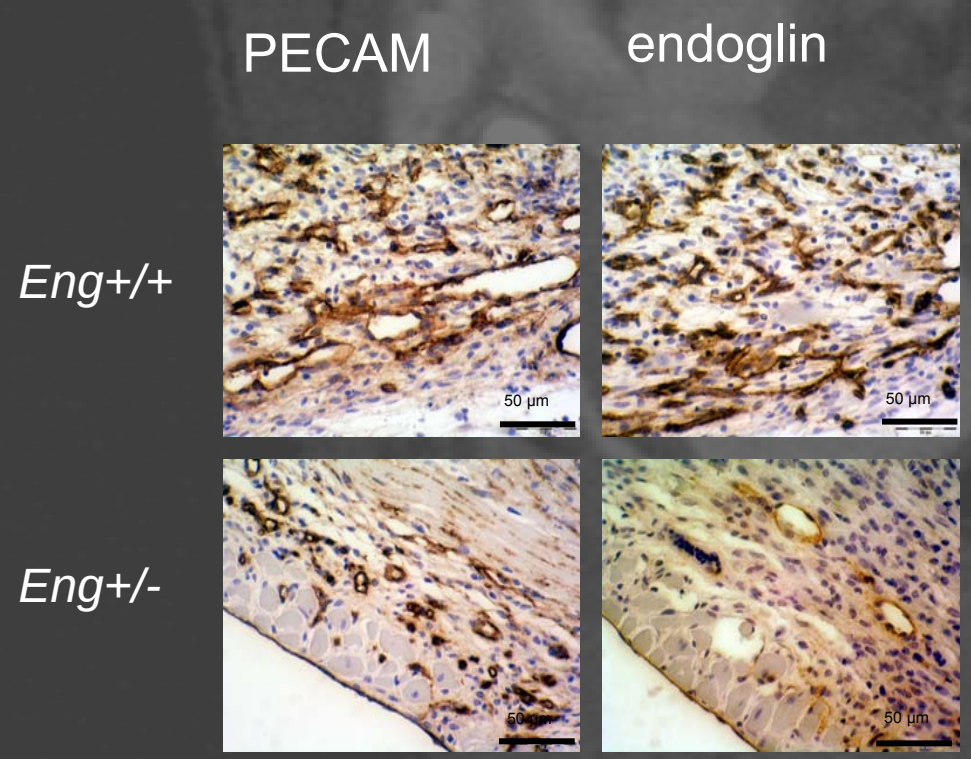
- Heart function: mouse MRI (1w, 4w)
- Statistical analysis: Mann-Whitney U test

Endoglin expression



- Endoglin upregulated in neoangiogenic vessels formed after MI (human and mouse)

Endoglin and neovascularization



Non-infarcted myocardium: no difference between *Eng*$^{+/-}$ and *Eng*$^{+/+}$

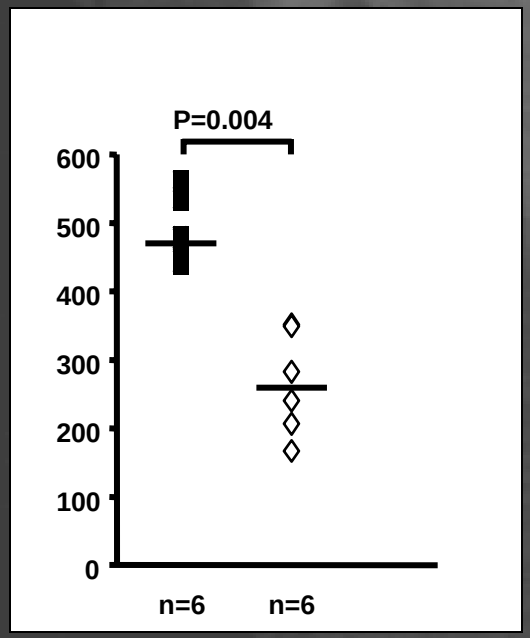
- Reduced upregulation of endoglin and neoangiogenesis in *Eng*$^{+/-}$ mice after MI
- CD45, CD68, Mac-3: no difference

Functional consequences

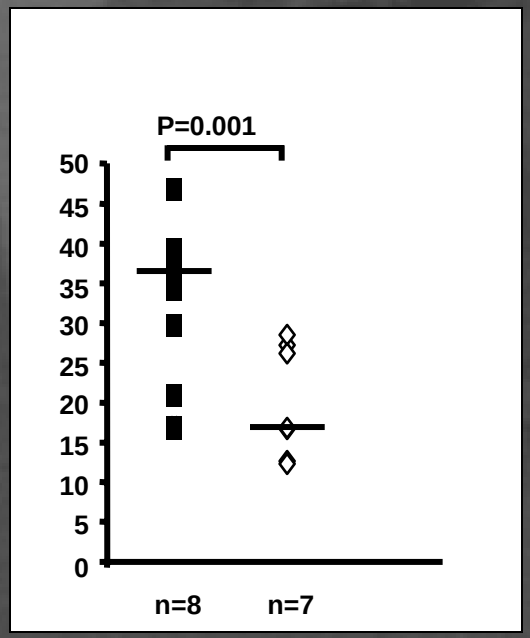
■ *Eng*$^{+/+}$

◆ *Eng*$^{+/-}$

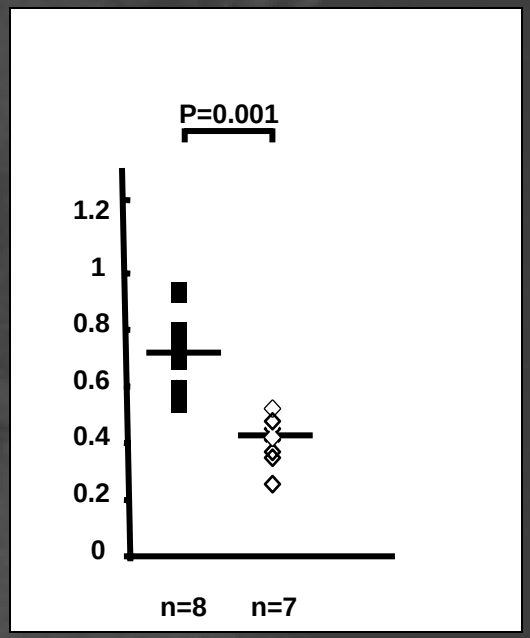
Vessels / mm^2



Ejection fraction (%)

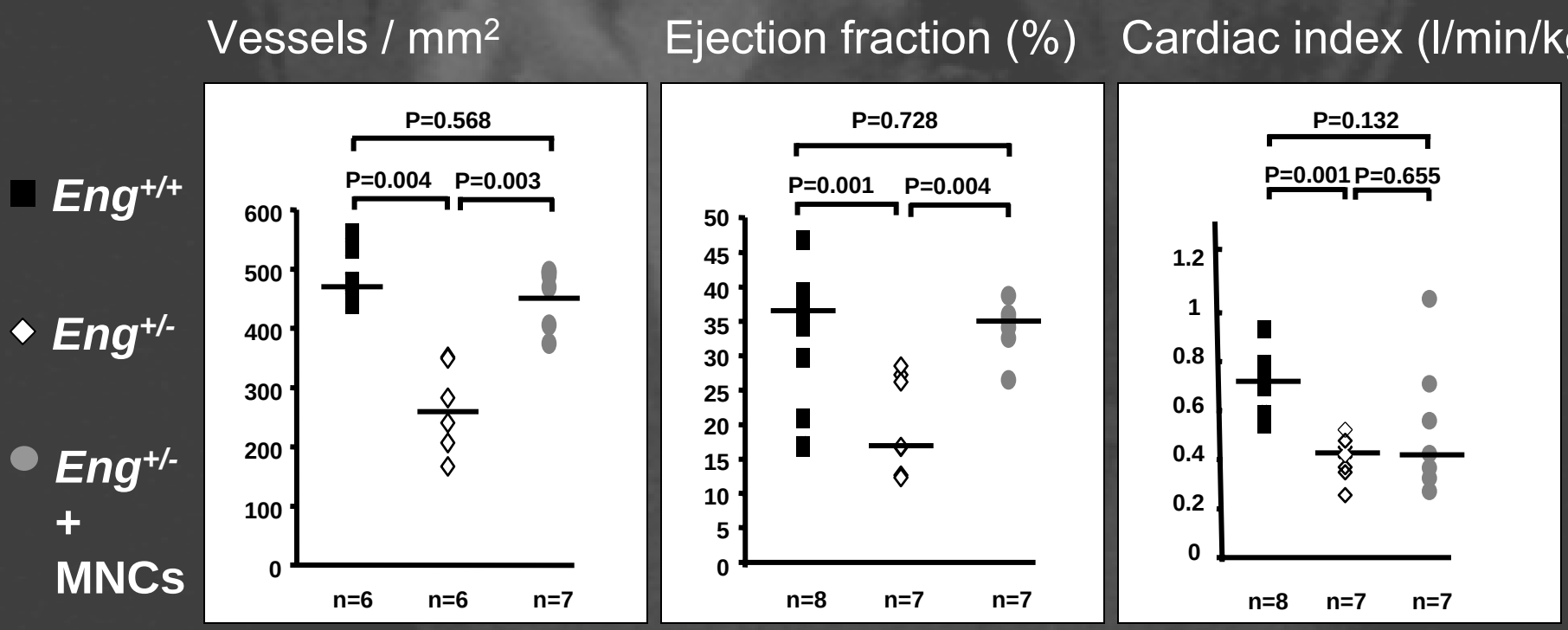


Cardiac index (l/min/kg)



- Neoangiogenesis defect in *Eng*+/- mice post-MI associated with impaired heart function

Functional consequences

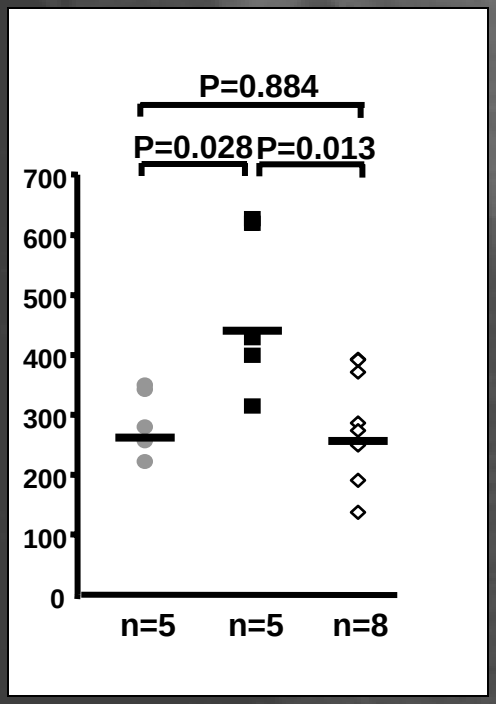


- Neoangiogenesis defect in *Eng*+/- mice post-MI associated with impaired heart function
- Partial rescue by injection of healthy MNCs

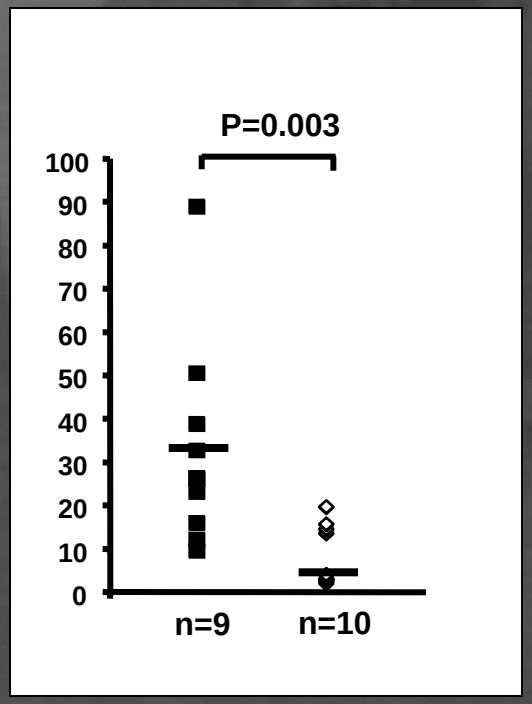
MNC defects

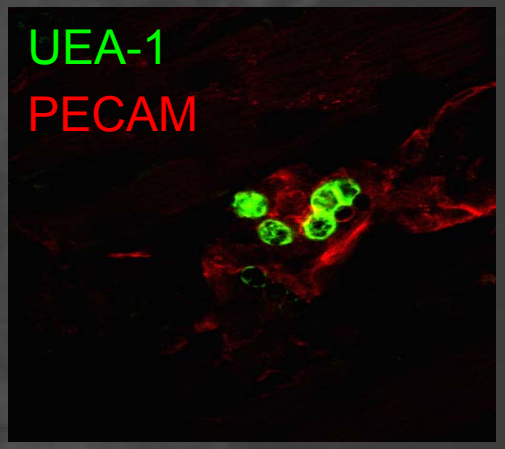
- Healthy donor MNC
- HHT1 patient MNC
- PBS

Vessels / mm^2



UEA-1 / mm^2





- HHT1-MNCs fail to stimulate neoangiogenesis and to accumulate in the infarct region of *Eng*+/+ mice

Conclusion

- Defective vascular repair –mediated by MNCs- is a significant component of the etiology of HHT1
- This may explain disease heterogeneity, since exposure to vascular damage or inflammation varies between patients
- In general, MNC characteristics in any patient may affect their efficiency of vascular repair

Acknowledgements

- Hubrecht Laboratory
  - Christine Mummery
  - Franck Lebrin
  - Sander van den Driesche
  - Alie Feijen
  - Mariette Driessens
- Heart Lung Center Utrecht
  - Pieter Doevendans
  - Marie-José Goumans
  - Simone Post
  - Maurits Jansen
  - Cees van Echteld
- St Antonius Hospital Nieuwegein
  - Cees Westerman
  - Repke Snijder
  - Johannes Mager
- Leiden University Medical Center
  - Peter ten Dijke
- University of Newcastle
  - Helen Arthur